New ligands of the histamine H3 receptor

Vollinga, R.C.

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Synthesis, Structure Activity Relationships and Molecular Pharmacology

Roeland C. Vollinga

VRIJE UNIVERSITEIT

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ACADEMISCH PROEFSCHRIFT

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De Boelelaan 1105

door

Roeland Christiaan Vollinga

geboren te 's Gravenhage

Promotor	:	prof.dr H. Timmerman
Copromotor	:	dr W.M.P.B. Menge
Referent	:	prof.dr G.J. Durant

In practical organic chemistry,
the precipitate is often the solution.

The investigations described in this thesis were for the major part performed at the Leiden/Amsterdam Center for Drug Research, Division of Medicinal Chemistry, Department of Pharmacochemistry, Vrije Universiteit Amsterdam,
De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands.
A part of the synthetic work described in chapter 10 was performed at the Department of Organic Pharmaceutical Chemistry, Uppsala Biomedical Centre, Uppsala University, Uppsala, Sweden (ULLA partner).

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Chapter 1

General Introduction

Introduction

Most people associate histamine with allergies, like rashes, hayfever or asthma. Although histamine is not the only mediator in these hypersensitive reactions of the body, "anti-histaminics" have been prescribed successfully in the treatment of many allergic disorders.
The physiological effects of histamine, like smooth muscle contraction, increased capillary permeability and stimulation of gastric acid secretion, were first described by Sir Henry Dale and his colleagues in 1910,[1] reporting the effect of histamine injections, similar to the effects observed in many allergic conditions. The following search for compounds antagonising this harmful action of histamine, led to the discovery of the first "anti-histaminics".[2,3] and many more potent antagonists have been synthesized since.
The observation that certain physiological effects of histamine, like gastric acid secretion and cardiac chronotropism, could not be blocked by any of the available "anti-histaminics", prompted Ash and Schild to postulate histamine receptor diversity. They defined the histamine receptors, which can be blocked by the "anti-histaminics" as H_1 receptors. The other histamine receptor subtype, evoking gastric acid secretion and cardiac chronotropism was defined as the H_2 receptor in 1972 by Black and Co-workers with the development of the selective antagonist burimamide.[4]

The existence of a third histamine receptor subtype, inhibiting the synthesis and release of histamine, located presynaptically in histaminergic nerve endings in rat cerebral cortex, was suggested in 1983 by Arrang *et al.*[5] Confirmation of the existence of this new histamine receptor subtype was provided by the development of the H_3 selective agonist (R)-α-methylhistamine and the H_3 selective antagonist thioperamide.[6] The H_3 receptor has since been shown to play an important regulatory role in the release of other neurotransmitters in the central nervous system[7-10] and in peripheral organs.[11-16]

For the histamine H_1- and H_2 receptors, many potent and selective ligands have been prepared. Whereas H_1 agonists are only desired for fundamental pharmacological research, H_1 antagonists are applied therapeutically in allergic conditions. The H_2 antagonists have become important, mainly in the treatment of peptic ulcers and H_2 agonists have been suggested as cardiac stimulants.

The role of histamine H_3 ligands as potential therapeutical agents, is under investigation, but more potent, selective and less toxic H_3 ligands than currently available, are still needed.[17]

Scope of this Thesis

The aim of the research, described in this thesis, was the synthesis of new histamine H_3 ligands, especially meant for SAR- and molecular pharmacology studies on the histamine H_3 receptor. This was mainly achieved by the preparation of derivatives of the endogenous H_3 agonist histamine and the H_3 antagonists burimamide and thioperamide. These compounds can be useful in the further characterisation of this receptor and its role in physiology.

The structural requirements for H_3 agonists or H_3 antagonists are discussed in chapter 2. In this chapter, an overview is presented of the most important compounds with histamine H_3 activity, that have been reported in literature so far. Several new compounds that have been synthesized recently in our own laboratories and which will be discussed in detail in subsequent chapters, have been included, in order to provide an overview, as complete as possible.
For the determination of the H_3 activity of the derivatives that we have prepared, an *in vitro* assay was developed. This functional test system is based on the inhibitory effect of H_3 agonists on the electrically evoked cholinergic contractions of a guinea pig intestine preparation. This simple test system was used for the rapid screening of all our newly synthesized compounds and is described, together with an overview of other possible H_3 *in vitro* assays, in chapter 3.
A suitable route for the synthesis of 4(5)-substituted imidazole derivatives was developed, since our target compounds all comprehended such a moiety. Potential synthesis routes, and the route of our choice, are discussed in chapter 4.
In chapter 5, the synthesis of 4(5)-(ω-aminoalkyl)-imidazoles is discussed, using a new route, based on the addition of a suitable bifunctional electrophile to a 5-lithiated, 1,2-diprotected imidazole. These derivatives can be regarded as homologues of histamine, but can also function as precursors for many other histaminergics. With this route other functionalized 4(5)-alkylimidazoles can be prepared as well.
The synthesis and identification of a new, potent and selective agonist for the H_3 receptor is presented in chapter 6. This compound, immepip, can be useful as a pharmacological tool, but also, because of its distinctive structure, for SAR- and Molecular Modelling studies.

The influence of alkyl chain length variation on the histamine H_3 receptor activity of homologues of histamine is described in chapter 7. A series of histamine homologues was prepared with an alkyl chain length varying from one methylene group to ten methylene groups. Elongation of the alkyl chain to a pentylene chain, led to a remarkable new potent and selective H_3 antagonist, impentamine.

Another class of selective H_3 antagonists was obtained by the synthesis of a large series of burimamide analogues, as well. This series is discussed in chapter 8.

Analogues of immepip and thioperamide have also been prepared. The synthesis routes, employing different electrophiles are discussed in chapter 9. These compounds were mostly much less active than there corresponding parent compounds, stressing the specific structural demands of H_3 ligands.

For the development of a theoretical model, using molecular modelling studies, an H_3 agonist with a small, rigid structure is required. A suitable compound for this purpose seems to be cyclopropylhistamine (4(5)-(2-aminocyclopropyl)-1*H*-imidazole).This compound has been described to be a potent H_3 agonist in a Patent Application.[18] Since the H_3 receptor proved to be stereoselective and it is unknown, which of the four possible diastereoisomers of cyclopropylhistamine is most active, part of the research was focused on the preparation of these isomers. The possible use of cyclopropylhistamine as a template for an H_3 receptor activation model, will be discussed in chapter 10, together with the synthesis of *trans*-cyclopropylhistamine and the attempts of preparing its *cis*-isomer.

Chapter 2

Structural Requirements for Histamine H_3 Receptor Ligands: an Overview

Introduction

The presence of histamine in the brain was already reported in 1919[19] and in 1959 it was demonstrated that histamine was synthesized their locally.[20] However, it lasted until 1975 before the role of histamine as a neurotransmitter was first described.[21] With the help of the available selective H_1- and H_2 ligands, the occurrence of H_1- and H_2 receptors in the brain was soon reported.[22,23]

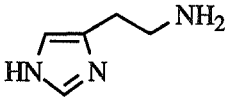
In 1983 it was observed that histamine inhibited its own K^+-stimulated release in rat cortical brain slices.[5] Since this effect could not be blocked by the available H_1 antagonists and only by some H_2 antagonists (but with a different potency than found in reference H_2 receptor mediated responses), it was suggested that these actions of histamine were mediated by a third histamine receptor subtype. This receptor was described to be located presynaptically on histaminergic nerve endings, regulating the release of histamine, by a negative feedback (autoreceptor). Confirmation of the existence of this new histamine receptor subtype was provided by the development of the H_3 selective agonist (R)-α-methylhistamine and the H_3 selective antagonist thioperamide.[6] The histamine H_3 receptor has since been shown, not only to inhibit the synthesis and release of histamine, but to play an important regulatory role in the release of other neurotransmitters (e.g. serotonin, acetylcholine, noradrenaline) in the central nervous system[7-10] and in the periphery[11-16] as well (heteroreceptor).

Many potent and selective ligands have been prepared for the histamine H_3 receptor, since its discovery. In this chapter, an overview is presented of the most important compounds displaying histamine H_3 activity, that have been reported in literature up to now. Several new compounds that have been synthesized recently in our own laboratories and which will be discussed in the following chapters of this thesis, have been included, in order to provide an overview, as complete as possible. The activities of the compounds, are all represented by their corresponding pD_2 cq pA_2 value, for easy comparison. However, since the H_3 activity of the compounds have been tested on different assays, it is not always possible to compare the biological activity directly (see chapter 3, where different H_3 test systems are discussed). Therefore each activity is represented with a reference to the test system that was used.

Histamine H_3 Agonists

Derivatives of histamine as H_3 agonists

Since histamine (**1**) itself is already remarkably active on the histamine H_3 receptor at very low concentrations, it is not surprising that most potent H_3 agonists, have been derived from it's structure.



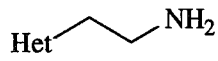
1, histamine

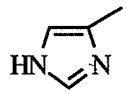
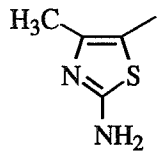
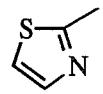
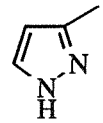
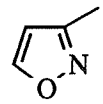
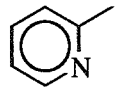
The 4(5)-alkyl-imidazole moiety in the structure of histamine (**1**), has proven to be essential for H_3 agonism. Replacement of the imidazole ring by other heterocycles, resulted in compounds without agonistic activity on the histamine H_3 receptor, although the H_2 agonist amthamine (**2**) has been described to be a very weak, but full H_3 agonist (see Table 1).

If the 2-aminoethylene chain of histamine is connected to the 2-position of the imidazole ring instead of the 4(5)-position, all activity is lost (see table 2).

Substituents on the imidazole ring are not allowed for agonistic activity. From Table 3 it is obvious that alkylation of the imidazole ring of histamine in any position, leads to a strong decrease in agonistic activity. This emphasizes the distinct structural demands of this particular histamine receptor, since methylation of the 2-position of histamine (2-methylhistamine, **13**) leads to a rather selective H_1 agonist and 4(5)-methylhistamine (**18**) is an H_2 agonist.

Table 1. Histamine H_3 Receptor activity of some histamine analogues, in which the imidazole ring is replaced by other heterocycles.



No.	Compound	Het	pD_2 value[a]	Ref.
1	histamine		7.4[b] (7.3[c])	5,13,24
2	amthamine		4.7[d]	25
3	2-(2-aminoethyl)-thiazole		< 3.0	5
4	3(5)-(2-aminoethyl)-pyrazole		< 4.0	26
5	3-(2-aminoethyl)-isoxazole		< 4.0	26
6	2-(2-aminoethyl)-pyridine		< 4.0	26

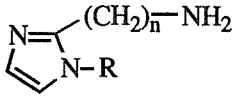
[a] Determined by evaluation of the influence of the compound on K^+-stimulated [^{3}H]-histamine release on rat cortex, as described by Arrang *et al.*[5]

[b] Same pD_2 value, determined by evaluation of the influence of the compound on electrically-stimulated [^{3}H]-histamine release on rat cortex, as described by Van der Werf *et al.*[27]

[c] Determined by evaluation of the influence of the compound on electrically evoked, NANC conctractions of guinea pig intestine preparations, as described by Menkveld *et al.*[13]

[d] Determined by evaluation of the influence of the compound on electrically evoked, cholinergic conctractions of guinea pig intestine preparations, as described by Vollinga *et al.*[15]

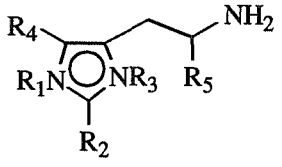
Table 2. Histamine H_3 activity of some 2-substituted ω-aminoalkyl imidazoles.



No.	Compound	n	R	pD_2 value[a]	Ref.
7	2-(2-aminoethyl)imidazole	2	H	< 4.0	26
8	2-(3-aminopropyl)imidazole	3	H	< 4.0	26
9	2-(4-aminobutyl)imidazole	4	H	< 4.0	26
10	1-methyl-2-(2-aminoethyl)imidazole	2	CH_3	< 4.0	26
11	1-methyl-2-(3-aminopropyl)imidazole	3	CH_3	< 4.0	26

[a] Determined by evaluation of the influence of the compound on K^+-stimulated [^{3}H]-histamine release on rat cortex, as described by Arrang *et al.*[5]

Table 3. Histamine H_3 Receptor activity of several ring substituted histamine analogues.



No.	Compound	R_1	R_2	R_3	R_4	R_5	pD_2 value[a]	Ref.
12	N^τ-methylhistamine	CH_3	H	H	H	H	< 6.0	5
13	2-methylhistamine	H	CH_3	H	H	H	< 4.3	5
14	2-ethylhistamine	H	C_2H_5	H	H	H	< 4.0	26
15	2-*n*-propylhistamine	H	C_3H_7	H	H	H	< 4.0	26
16	2-phenylhistamine	H	C_6H_5	H	H	H	< 4.0	26
17	N^π-methylhistamine	H	H	CH_3	H	H	< 6.0	5
18	4(5)-methylhistamine	H	H	H	CH_3	H	< 3.0	5
19	(R)-α,N^τ-methylhistamine	CH_3	H	H	H	(R)-CH_3	4.3	26
20	(±)-5(4),α-dimethylhistamine	H	H	H	CH_3	CH_3	< 4.0	26

[a] Determined by evaluation of the influence of the compound on K^+-stimulated [^{3}H]-histamine release on rat cortex, as described by Arrang *et al.*[5]

Whereas the imidazole ring may not be replaced or substituted for agonistic activity on the H_3 receptor, modifications of the side chain of histamine have resulted in some very potent and selective H_3 agonists (Table 4 and Table 5). Especially the introduction of a methyl group on the ethylene chain of histamine resulted in compounds that are more potent on the H_3 receptor. Upon methylation, the stereoselective character of the H_3 receptor becomes apparent, since the (R)-enantiomer of α-methylhistamine **21** is about 100 times more potent than its (S)-enantiomer **22** on the rat cortex. Because of its potency and selectivity (about 20,000 times more potent on the H_3 receptor than on the H_1- and H_2 receptors),[6] (R)-α-methylhistamine is most often used as the standard agonist for pharmacological assays related to histamine H_3 receptors.

Replacement of the methyl group by a chloromethyl- or a hydroxymethyl group leads to a reduction of activity, which might be the result of steric hindrance (**23-26**). However, also these compounds display a large difference in activity between the (R)- and the (S)-enantiomer (compare **23** and **25** with **24** respectively **26**). The eutomer (most active enantiomer) has the same absolute configuration as (R)-α-methylhistamine (**21**).

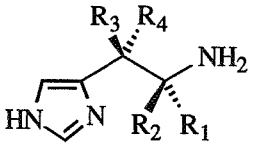
The reported weak antagonistic activity of (±)-α-aminomethylhistamine (**30**) may be explained differently, since this compound can also be regarded as an analogue of the H_3 antagonist homohistamine (**109**) (see histamine H_3 antagonists, table 14).

The introduction of a methyl group on the β-position of the ethylene chain of histamine results in a potent H_3 agonist as well ((±)-β-methylhistamine, **31**) and also here an increase of the van der Waals volume leads to a decrease of activity, since (±)-β-ethylhistamine (**32**) is a very weak agonist.

Whereas large substituents on the ethylene chain of histamine are not allowed (see also the cyclohexylhistamines **39** and **40**), the histamine derivative with two methyl groups on the α-position **33** is still a good agonist. β,β-Dimethylhistamine (**37**) however, results in a very weak agonist, suggesting that there is not enough space in the 'active site' of the receptor, to accommodate two methyl groups on the β-position.

If both the α- and the β-position are methylated, (R)α,(S)β-dimethylhistamine (**27**) is the active diastereoisomer. This compound is equipotent as (R)-α-methylhistamine (**21**) on the rat cortex and (S)α,(R)β-dimethylhistamine (**28**) is equipotent as (S)-α-methylhistamine (**22**), indicating that the stereochemistry of the α-methyl group largely determines the activation of the receptor. In addition to its high potency, (R)α,(S)β-dimethylhistamine (**27**) is also highly selective, displaying an activity profile that is about 100,000-fold more active at the H_3 receptor than at the H_1- and H_2 receptors.[28]

Table 4. Agonistic activity of several optically pure substituted histamine analogues at the H_3 receptor.

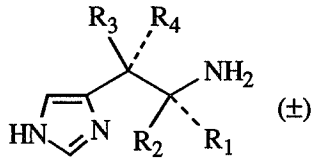


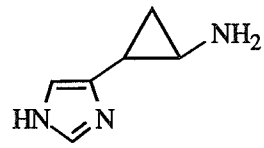
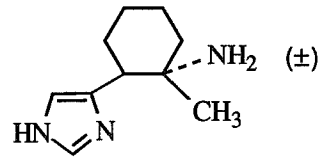
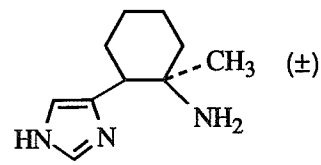
No.	Compound (HA=histamine)	R_1	R_2	R_3	R_4	pD_2 value[a]	Ref.
21	(R)-α-methylHA	CH_3	H	H	H	8.4, 7.8[b]	6,15
22	(S)-α-methylHA	H	CH_3	H	H	6.3	6
23	(S)-α-chloromethylHA	CH_2Cl	H	H	H	5.9	26
24	(R)-α-chloromethylHA	H	CH_2Cl	H	H	4.7	26
25	(S)-α-hydroxymethylHA	CH_2OH	H	H	H	5.7	26
26	(R)-α-hydroxymethylHA	H	CH_2OH	H	H	4.2	26
27	(R)α,(S)β-dimethylHA	CH_3	H	CH_3	H	8.5	29
28	(S)α,(R)β-dimethylHA	H	CH_3	H	CH_3	6.5	29

[a] Determined by evaluation of the influence of the compound on K^+-stimulated [^{3}H]-histamine release on rat cortex, as described by Arrang *et al.*[5]

[b] Determined by evaluation of the influence of the compound on electrically evoked, cholinergic conctractions of guinea pig intestine preparations, as described by Vollinga *et al.*[15]

Table 5. Agonistic activity of several substituted histamine analogues at the H_3 receptor (unresolved mixture of enantiomers).



No.	Compound (HA=histamine)	R_1	R_2	R_3	R_4	pD_2 value[a]	Ref.
29	(±)–α-ethylHA	C_2H_5	H	H	H	5.0	29
30	(±)–α-aminomethylHA	CH_2NH_2	H	H	H	pA_2=5.3	26
31	(±)–β-methylHA	H	H	CH_3	H	7.7	29
32	(±)–β-ethylHA	H	H	C_2H_5	H	5.0	29
33	α,α-dimethylHA	CH_3	CH_3	H	H	7.6	29
34	(±)–α-methylhistidine	CH_3	CO_2H	H	H	5.8	26
35	erythro-α,β-dimethylHA	CH_3	H	CH_3	H	8.2	28,29
36	threo-α,β-dimethylHA	H	CH_3	CH_3	H	6.7	28,29
37	β,β-dimethylHA	H	H	CH_3	CH_3	5.8	30
38	(±)–cyclopropylHA					8.0	18
39	(±)–*trans*-α-methyl-cyclohexylHA					$pK_i < 5.7$[b]	31
40	(±)–*cis*-α-methyl-cyclohexylHA					$pK_i < 5.7$[b]	31

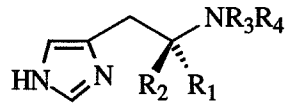
[a] Determined by evaluation of the influence of the compound on K^+-stimulated [^{3}H]-histamine release on rat cortex, as described by Arrang *et al.*[5]

[b] Determined on guinea pig brain tissue, measuring the displacement of [^{3}H] N^{α}-methylhistamine, as described by Korte *et al.*[32]

Another interesting reported histamine analogue with good H_3 agonistic activity is cyclopropylhistamine (4(5)-(2-aminocyclopropyl)-1*H*-imidazole) (**38**).[33] This molecule has been reported as a full H_3 agonist in a Patent Application[18] and might be very useful for structure-activity relationship and molecular modelling studies, because of its small and rigid structure. Unfortunately nothing more has been reported in literature about this compound. It is likely that the sample as described in the Patent Application, consisted of a mixture of all four possible stereoisomers of cyclopropylhistamine (**38**), because a non-stereoselective synthesis route was used for its preparation. For the use of cyclopropylhistamine (**38**) in further SAR or molecular modelling studies, the determination of the eutomer of cyclopropylhistamine is obligatory (see also Chapter 10).

In addition to alkylations on the side chain of histamine that have been performed, resulting in potent and selective agonists, the amino group has also been alkylated (see table 6). The introduction of one methyl group on the amino group results in a potent H_3 agonist and even two methyl groups are tolerated for activity (although there is a large difference in activity between N^{α}-methylhistamine (**41**) and N^{α},N^{α}-dimethylhistamine (**42**) on the electrically stimulated [^{3}H]-histamine release assay). The introduction of larger substituents on the amino group leads to a decrease in activity. Whereas N^{α}-ethylhistamine (**43**) is still a full agonist, the incorporation of the amino group in a pyrrolidine ring results in a partial agonist (compound, **44**) and introduction of a propyl group leads to a complete loss of activity (N^{α}-propylhistamine, **45**).
Intriguing is the observation that (R)-α-methylhistamine (**21**) and N^{α}-methylhistamine (**41**) are both potent H_3 agonists, but that (R)α,N^{α}-dimethylhistamine (**46**) is a weak agonist. This could mean that there is not enough space in the receptor, to tolerate substituents on both the α-position and the amino group. Another possible explanation may be that (R)-α-methylhistamine (**21**) and N^{α}-methylhistamine (**41**) are in a different conformation when binding to the receptor or that (R)α,N^{α}-dimethylhistamine (**46**) is in a different conformation than (R)-α-methylhistamine (**21**) and N^{α}-methylhistamine (**41**). However it must be noted that the trend of a decrease in activity, going from (R)-α-methylhistamine (**21**) to its (S)-enantiomer **22**, can also be observed between the enantiomers of α,N^{α}-dimethylhistamine (**46** and **47**), as well as between the enantiomers of α-chloromethyl-N^{α}-methylhistamine (**48** and **49**). Introduction of the more hydrophilic α-hydroxymethyl group to N^{α}-methylhistamine is clearly not tolerated and both enantiomers (**50** and **51**) are inactive.

Table 6. H_3 Agonistic activity of several N^{α}-alkyl substituted histamine analogues.



No.	Compound	R_1	R_2	R_3	R_4	pD_2 value[a]	Ref.
41	N^{α}-methylhistamine	H	H	CH_3	H	7.8[b] (8.7[c])	5,15,24
42	N^{α},N^{α}-dimethylhistamine	H	H	CH_3	CH_3	7.6 (7.3[c])	5,24
43	N^{α}-ethylhistamine	H	H	C_2H_5	H	7.1	34
44	4(5)-(2-pyrrolidin-ethyl)imidazole	H	H	$—(CH_2)_4—$		6.0[d]	6
45	N^{α}-propylhistamine	H	H	C_3H_7	H	< 5.2	34
46	(R)α,N^{α}-dimethyl-histamine	CH_3	H	CH_3	H	5.8	35
47	(S)α,N^{α}-dimethyl-histamine	H	CH_3	CH_3	H	4.3	35
48	(S)α-chloromethyl-N^{α}-methylhistamine	CH_2Cl	H	CH_3	H	5.3	35
49	(R)α-chloromethyl-N^{α}-methylhistamine	H	CH_2Cl	CH_3	H	3.0	35
50	(S)α-hydroxymethyl-N^{α}-methylhistamine	CH_2OH	H	CH_3	H	3.2	26
51	(R)α-hydroxymethyl-N^{α}-methylhistamine	H	CH_2OH	CH_3	H	3.0	26

[a] Determined by evaluation of the influence of the compound on K^+-stimulated [^{3}H]-histamine release on rat cortex, as described by Arrang *et al.*[5]

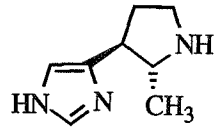
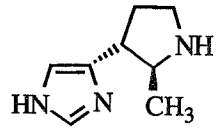
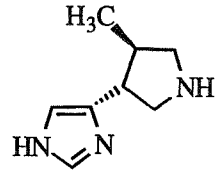
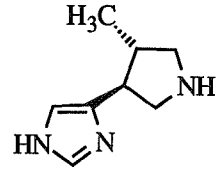
[b] Same pD_2 value, determined by evaluation of the influence of the compound on electrically evoked, cholinergic conctractions of guinea pig intestine preparations, as described by Vollinga *et al.*[15]

[c] Determined by evaluation of the influence of the compound on electrically-stimulated [^{3}H]-histamine release on rat cortex, as described by Van der Werf *et al.*[27]

[d] Partial agonist ($\alpha = 0.6$).

Recently a new series of histamine analogues has been described, in which the structure of histamine is partly incorporated in a ring structure (see table 7 and table 8). In this series, variations in ring size and substituents were performed. SCH 49648 (**52**), which can be regarded as a rigid analogue of (R)-α-methylhistamine (**21**) is still reasonably active, although less potent as (R)-α-methylhistamine itself. Moreover it has been reported, that SCH 49648 (**52**) and SCH 50971 (**54**) were both as effective as (R)-α-methylhistamine (**21**) *in vivo* in the inhibition of an electrically induced CNS hypertensive response.[31]

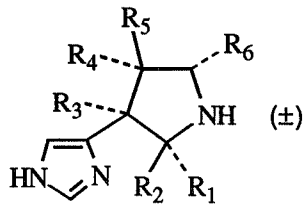
Table 7. Histamine H_3 activity of some optically pure ring-closed histamine analogues.

No.	Compound	Structure		pD_2 value[a]	pK_i value[b]	Ref.
52	SCH 49648		(+)-[2R,3S]	7.1	8.5	31
53	SCH 49647		(-)-[2S,3R]		7.5	31
54	SCH 50971		(+)-[3R,4R]	7.5	8.6	31
55	SCH 50972		(-)-[3S,4S]		7.5	31

[a] Determined by evaluation of the influence of the compound on electrically evoked, cholinergic conctractions of guinea pig intestine preparations, as described by Vollinga *et al.*[15]

[b] Determined on guinea pig brain tissue, measuring the displacement of [^{3}H] N^{α}-methylhistamine, as described by Korte *et al.*[32]

Table 8. Histamine H_3 activity of some ring-closed histamine analogues (enantiomers unresolved).



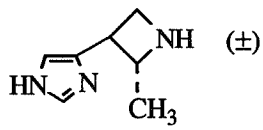
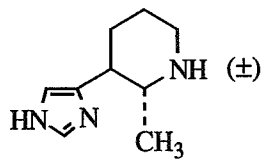
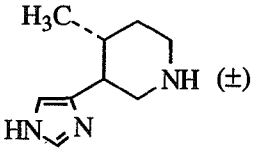
No.	R_1	R_2	R_3	R_4	R_5	R_6	pK_i value[a]	Ref.
56	H	H	H	H	H	H	8.5; 8.7	31,36
57	CH_3	H	H	H	H	H	8.2;8.5	31,36
58	$CH_2C_6H_5$	H	H	H	H	H	< 5.8	31,36
59	H	CH_3	H	H	H	H	6.8	31,36
60	H	$(CH_2)_2C_6H_5$	H	H	H	H	6.2	36
61	H	H	CH_3	H	H	H	6.8	31,36
62	H	H	H	CH_3	H	H	8.1;8.3	31,36
63	H	H	H	C_2H_5	H	H	6.8; 6.4	31,36
64	H	H	H	C_5H_{11}	H	H	6.3	31,36
65	H	H	H	H	CH_3	H	8.5	31
66	H	H	H	H	C_5H_{11}	H	7.1	31,36
67	H	H	H	H	H	CH_3	7.2;7.1	31,36
68	CH_3	CH_3	H	H	H	H	7.2;6.2	31,36
69	CH_3	H	H	CH_3	H	H	< 5.7	31
70	CH_3	H	H	H	CH_3	H	6.7	31
71	H	H	H	CH_3	CH_3	H	7.0	31
72							8.2	31,36
73							< 5.7	31

Table 8. (Continued).

No.	Structure	pK_i value[a]	Ref.
74		6.4	31

[a] Determined on guinea pig brain tissue, measuring the displacement of [^{3}H] N^{α}-methylhistamine, as described by Korte *et al.*[32]

When comparing affinities, SCH 49648 (**52**) (same absolute configuration as (R)-α-methylhistamine (**21**)) can be regarded as the eutomer and SCH 49647 (**53**) as the distomer.
Whereas large substituents on the β-position of the ethylene chain of histamine were not tolerated (see Table 5), it is remarkable that SCH 50971 (**54**), which can also be regarded as an analogue of β-ethylhistamine (**32**) is even slightly more active than SCH 49648 (**52**). This means that large substituents on the β-position are tolerated, but that the stereochemistry on this position is very critical for H_3 agonism.
Another intriguing observation is that the absolute configurations of the 3-position of the pyrrolidine ring of SCH 49648 (**52**) and SCH 50971 (**54**) are different, but the affinities of both compounds for the H_3 receptor are comparable. This would indicate totally different orientations of both compounds when binding to the receptor.

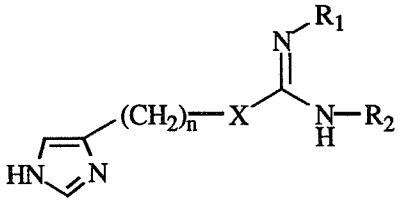
When comparing the affinities of the unresolved (mixture of enantiomers) ring-closed histamine analogues (Table 8), an increase of the ring size from a five membered ring to a six membered ring (**73**, **74**) results in a large decrease in affinity (compare **57** with **73**). A decrease of ring size to a four membered ring (**72**) leads to a compound with a high affinity for the H_3 receptor, but less selectivity, since H_1 activity has been reported for **72**.[31] Also for the large series of five membered rings, the introduction of methyl substituents seems to be essential for selectivity, since the nor-methyl analogue (**56**) exhibits H_1 activity as well.[31]
The introduction of larger substituents on the pyrrolidine ring results in a strong decrease in activity. The compounds with the highest affinities have a methyl group on the 2-position (**57**) or at the 4-position (**62**), *trans* (not on the same site of the pyrrolidine ring) to the imidazole ring. However the introduction of two methyl groups, one on the 2-position and the other at the 4-position, both *trans*-orientated, relatively to the imidazole ring (compound, **69**), is not tolerated, suggesting a differ-

ent binding orientation between the 2-methyl- (**57**) and the 4-methyl derivative (**62**). The derivative with a *cis* orientated methyl group relatively to the imidazole ring on the 4-position (**65**) has a high affinity as well, but has also been described to display H_1 activity.[31]

Derivatives of imetit as H_3 agonists

The replacement of the amino group of histamine by an isothiourea group has resulted in the very potent and selective H_3 agonist imetit (**75**).

Table 9. Histamine H_3 activities of some analogues of imetit.



No.	Compound	n	X	R_1	R_2	pD_2 value	Ref.
75	imetit	2	S	H	H	8.1[a,b] (9.0[c])	37-40
76	SKF 91606	2	CH_2	H	H	9.0[b]	38
77	VUF 83100	2	NH	H	H	6.3[a] (7.4[d,e])	37,41
78	VUF 8621	2	S	CH_3	H	7.3[a] (7.8[c])	37,40
79	VUF 8973	2	S	CH_3	CH_3	pA_2 = 6.6[a] (7.3[c])	37,40
80	VUF 8321	1	S	H	H	5.2[a]	37
81	VUF 8328	3	S	H	H	pA_2 = 8.0[a]	37

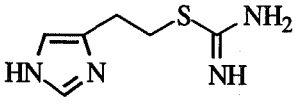
[a] Determined by evaluation of the influence of the compound on electrically evoked, NANC conctractions of guinea pig intestine preparations, as described by Menkveld *et al.*[13]

[b] Determined by evaluation of the influence of the compound on electrically evoked, cholinergic conctractions of guinea pig intestine preparations, as described by Vollinga *et al.*[15]

[c] Determined by evaluation of the influence of the compound on K^+-stimulated [^{3}H]-histamine release on rat cortex, as described by Arrang *et al.*[5]

[d] Determined by evaluation of the influence of the compound on electrically-stimulated [^{3}H]-histamine release on rat cortex, as described by Van der Werf *et al.*[27]

[e] Partial agonist (α = 0.5).

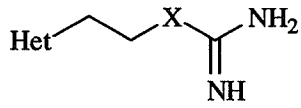


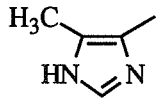
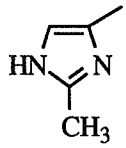
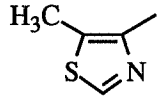
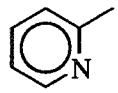
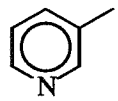
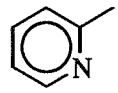
75, imetit

Several analogues of this compound have been prepared (see table 9). The sulphur atom of the isothiourea group of imetit (**75**), is not involved in an interaction with the H_3 receptor, since SKF 91606 (**76**) is even more potent. SKF 91606 (**76**) is the most potent H_3 agonist sofar, but has only be reported once.[38] Replacement of the isothiourea group of imetit by a guanidine group (VUF 83100, **77**) results in a drastic decrease of activity.

The introduction of substituents on the isothiourea group of imetit (**75**) is not very well tolerated for agonism, since the monomethylated imetit derivative VUF 8621 (**78**) is less active and the introduction of two methyl groups results in an antagonist VUF 8973 (**79**). Variation in the length of the alkyl chain is also not allowed for H_3 agonistic activity and VUF 8328 (**81**) is even a potent H_3 antagonist. Apparently the distance between the amidine system and the imidazole ring is very strict for H_3 agonism. The introduction of a methyl group on the 5-position of the imidazole ring of imetit leads to a weak agonist (VUF 9032, **82**) and a methyl group on the 2-position results in a very weak antagonist (VUF 8974, **83**). Replacement of the imidazole ring with other heterocycles results in inactive compounds (see table 10). Only VUF 8863 (**85**) has been reported a very weak partial agonist. These results indicate again the importance of the 4(5)-alkyl-imidazole moiety for H_3 agonism and the intolerance (probably sterical hindrance) for additional substituents on this ring.

Table 10. Histamine H_3 activities of analogues of imetit, in which the imidazole has been replaced by other heterocycles.



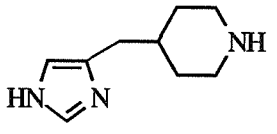
No.	Compound	Het	X	pD_2 value	Ref.
82	VUF 9032		S	6.0 ($\alpha = 0.9$)[a]	37
83	VUF 8974		S	$pA_2 = 5.4$[a]	37
84	VUF 8844		S	inactive[b]	41
85	VUF 8863		S	6.0 ($\alpha = 0.5$)[b]	41
86	VUF 8848		S	inactive[b]	41
87	VUF 8862		NH	inactive[b]	41

[a] Determined by evaluation of the influence of the compound on electrically evoked, NANC conctractions of guinea pig intestine preparations, as described by Menkveld *et al.*[13]

[b] Determined by evaluation of the influence of the compound on electrically-stimulated [^{3}H]-histamine release on rat cortex, as described by Van der Werf *et al.*[27]

Derivatives of immepip as H_3 agonists

Recently a new potent and selective H_3 agonist has been described, which is not an ethylene analogue of histamine (see Chapter 6).[42]

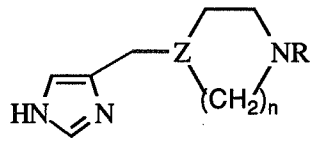


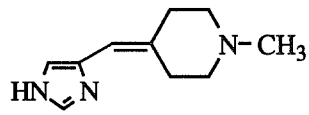
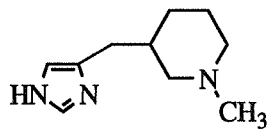
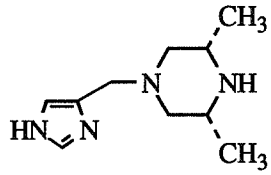
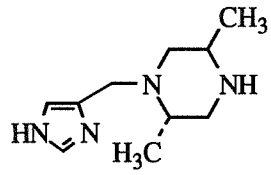
88, immepip

This compound, immepip (**88**) has a pD_2 value of 8.0 on the guinea pig intestine and is therefore equipotent as (R)-α-methylhistamine (**21**) and imetit (**75**) on the same assay,[15,42] but has a distinct structure.
The affinities of immepip (**88**) and some of its analogues as determined on guinea pig brain tissue (see table 11), have also been reported independently, however in a Patent Application only.[43] From table 11 it can be seen that immepip (**88**) has a high affinity for the H_3 receptor. Methylation of the amino group leads to a slight decrease in affinity (compound **91**). Replacement of the piperidine ring of immepip by a pyrrolidine ring however, results in a drastic reduction of affinity (compound **89**). It must be noted that this compound has not been resolved in its enantiomers. Surprisingly, methylation of the amino group of this pyrrolidine derivative **89**, results in a compound with a strongly increased affinity (compound **90**). The replacement of the piperidine ring by the synthetically more convenient piperazine ring (compound **92**), results in a ten fold decrease of affinity and the introduction of methyl substituents on the piperazine ring leads to a drastic loss of affinity.

Because immepip (**88**) has the highest affinity, it seems likely that it is also the most active H_3 agonist from this series. However, in order to draw conclusions for structure-activity relationship studies it is necessary to determine the H_3 activities of the analogues represented in table 11.

Table 11. Affinities of some analogues of immepip, determined on guinea pig brain tissue (enantiomers unresolved).



No.	Z	n	R	pK_i value[a]	Ref.
88[b]	CH	2	H	9.5	43
89	CH	1	H	7.0	36
90	CH	1	CH_3	8.5	36
91	CH	2	CH_3	9.1	43
92	N	2	H	8.3	44
93	N	3	H	7.1	36
94				7.9	43
95				6.7	43
96				< 5.2	44
97				< 5.2	44

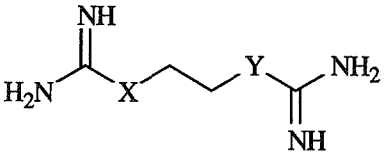
[a] Determined on guinea pig brain tissue, measuring the displacement of [^{3}H] N^{α}-methylhistamine, as described by Korte *et al.*[32] Values from a Patent Application.

[b] Immepip; pD_2 = 8.0 as determined by evaluation of the influence of the compound on electrically evoked, cholinergic conctractions of guinea pig intestine preparations, as described by Vollinga *et al.*[15]

Miscellaneous H_3 agonists

Obviously, the 4(5)-alkyl-imidazole moiety is essential for activation of the H_3 receptor, since this structural element is apparent in all potent H_3 agonists known at this time. The only non-imidazole derivatives known sofar, which have been reported to be weakly and partially active at the H_3 receptor are VUF 8863 (**85**) (see table 10) and VUF 8430 (**98**) (see table 12). If an interaction of either the isothiourea group or the guanidine group of VUF 8430 (**98**) is assumed, it is interesting to see that the compound with two guanidine groups **100** is inactive and that the bis-isothiourea derivative **99** is a weak antagonist. Apparently both functionality's are required for (partial) activation of the H_3 receptor by VUF 8430 (**98**). Based on these results it might be speculated that the guanidine group is capable of activating the receptor (although weakly), but that the isothiourea group of VUF 8430 (**98**) is required for the binding to the receptor. This could also explain why the guanidine derivative of imetit (VUF 83100, **77**) is much less active (see table 9), since the guanidine group is less capable of binding to the receptor.

Table 12. Histamine H_3 activity of some bifunctional amidine derivatives.



No.	Compound	X	Y	H_3 activity[a]	Ref.
98	VUF 8430	S	NH	$pD_2 = 5.9$ (α=0.5)	41
99	VUF 8332	S	S	$pA_2 = 6.0$	41
100	VUF 8309	NH	NH	inactive	41

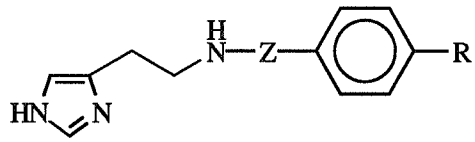
[a] Determined by evaluation of the influence of the compound on electrically-stimulated [^{3}H]-histamine release on rat cortex, as described by Van der Werf *et al.*[27]

Histamine H_3 Antagonists

Derivatives of histamine with H_3 antagonistic activity

From table 6 it is clear that large substituents on the amino group of histamine, diminish the H_3 agonistic activity and in table 13 some other N^{α}-substituted histamine derivatives are presented, which have been reported as weak H_3 antagonists. There is not a large influence of the length of the alkyl chain, separating the phenyl ring from the amino group. Apparently the position of this phenyl ring is not very important for antagonistic activity. However a ten-fold increase in antagonistic activity is observed if a 4-chloro group is introduced on the phenyl ring of **106**, indicating that lipophilicity plays a role in the binding of these compounds to the H_3 receptor (compound **107**).

Table 13 Histamine H_3 antagonistic activity of some N^{α} substituted histamine analogues.

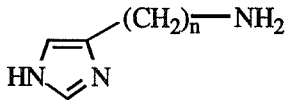


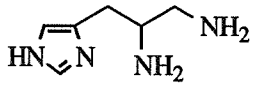
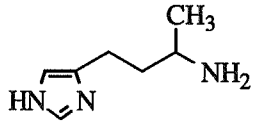
No.	Z	R	pA_2 value[a]	Ref.
101	$(CH_2)_2$	H	5.5	45
102	$(CH_2)_3$	H	6.2	45
103	$(CH_2)_4$	H	6.2	45
104	$(CH_2)_5$	H	5.7	45
105	$(CH_2)_2O$	H	6.4	45
106	$(CH_2)_2S$	H	5.6	45
107	$(CH_2)_2S$	Cl	6.6	45

[a] Determined by evaluation of the influence of the compound on K^+-stimulated [^{3}H]-histamine release on rat cortex, as described by Arrang *et al.*[5]

Elongation of the side chain of histamine (**1**) resulted in compounds with H_3 antagonistic properties (see table 14), with impentamine (**111**) as the most potent and selective H_3 antagonist from this series (see Chapter 7 for more details).

Table 14 Histamine H_3 antagonistic activity of some homologues of histamine, in which the length of the ω-aminoalkyl chain is varied.



No.	Compound	n	pA_2 value[a]	Ref.
108	VUF 8319	1	inactive	46
1	histamine	2	$pD_2 = 7.3$[b]	5
109	homohistamine	3	5.9	46
110	VUF 4701	4	7.7	46
111	impentamine	5	8.4	46
112	VUF 4732	6	7.8	46
113	VUF 4733	8	6.0	46
114	VUF 4734	10	6.0	46
30	(±)–α-aminomethyl-histamine		5.3[c]	26
115	(±)-α-methyl-homohistamine		$pD_2 < 4.0$[c]	26

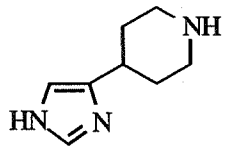
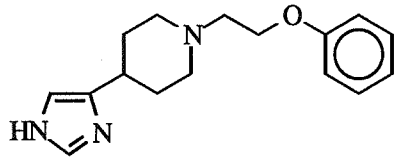
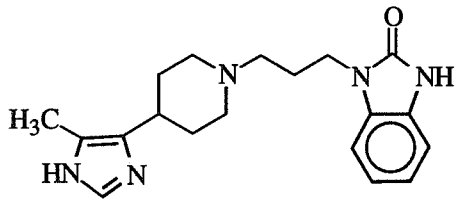
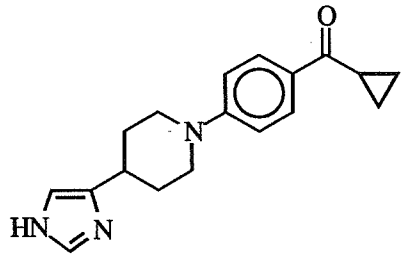
[a] Determined by evaluation of the influence of the compound on electrically evoked, cholinergic conctractions of guinea pig intestine preparations, as described by Vollinga *et al.*[15]

[b] Determined by evaluation of the influence of the compound on electrically evoked, NANC conctractions of guinea pig intestine preparations, as described by Menkveld *et al.*[13]

[c] Determined by evaluation of the influence of the compound on K^+-stimulated [^{3}H]-histamine release on rat cortex, as described by Arrang *et al.*[5]

The fact that homohistamine (**109**) is a weak antagonist, might explain why α-aminomethylhistamine (**30**, already mentioned in table 5) is a weak antagonist, since this compound can also be regarded as an analogue of homohistamine. For the same reason, α-methylhomohistamine (**115**), which has been reported to show no H_3 agonistic activity, might be a weak antagonist as well.

Table 15 Histamine H_3 antagonistic activity of some analogues of histamine with an aminopropylene chain, enclosed in a piperidine ring.

No.	Structure	pA_2 value[a]	Ref.
116		5.7 (6.2[b])	26,47
117		7.2	48
118		6.3	48
119		7.4	48

[a] Determined by evaluation of the influence of the compound on K^+-stimulated [^{3}H]-histamine release on rat cortex, as described by Arrang *et al.*[5]

[b] Determined by evaluation of the influence of the compound on electrically evoked, cholinergic conctractions of guinea pig intestine preparations, as described by Vollinga *et al.*[15]

The rigid piperidine analogue of homohistamine, **116** is still a weak antagonist, but coupling of a lipophilic moiety to the amino group leads to an increase of antagonistic activity (see table 15). Introduction of a methyl group on the 5-position of the imidazole ring, presumably results in a decrease of affinity, although compound **118** is slightly more potent than **116** on the rat cortex.

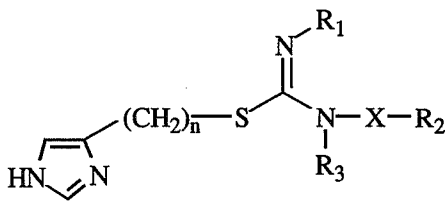
Derivatives of imetit with H_3 antagonistic activity

Also derivatives of the potent H_3 agonist imetit (**75**) resulted in some potent antagonists (see table 16). Substitution of the isothiourea group with more than one methyl group leads to moderately active H_3 antagonists. The introduction of a phenyl group, separated from the isothiourea group by an alkyl chain leads to good antagonists. The length of this alkyl spacer does not seem to be very strict. The introduction of a methyl group on the isothiourea group of the phenylethyl derivative VUF 9029 (**125**), does not influence the activity (see VUF 9051 (**126**)).

Elongation of the ethylene chain of imetit (**75**) however, has a drastic effect on the activity. VUF 8328 (**81**) is already a potent antagonist and the introduction of a phenyl group to the isothiourea group, separated by an alkyl spacer, leads to more potent H_3 antagonists. Replacement of a phenyl group by a non-aromatic cyclohexyl group (VUF 9163, **132**) has no effect on the H_3 antagonistic activity, suggesting that receptor binding by this part of the molecule is through a hydrophobic interaction and not a π-π interaction. This is verified by the introduction of a chloro group in the *para* position of the benzyl group, resulting in clobenpropit (**131**), the most potent H_3 antagonist reported sofar (pA_2 value of 9.9 at guinea pig ileum). This observation led to the development of the first selective radiolabelled H_3 antagonist [^{125}I]-iodophenpropit (**134**), which is currently used as a radioligand for histamine H_3 receptor binding studies.[49-51]

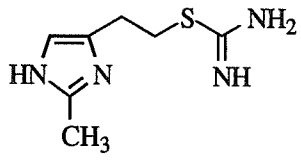
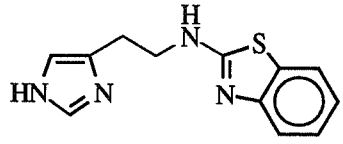
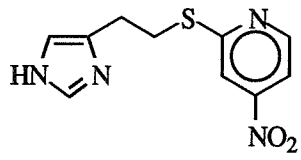
Methylation of the 2-position of the imidazole ring of imetit results in a very weak antagonist (VUF 8974, **83**), whereas 5-methylimetit (**82**, see table 10) is a weak agonist.

Table 16 Histamine H_3 antagonistic activity of several substituted analogues of imetit.



No.	Compound	n	R_1	X	R_2	R_3	pA_2 value[a]	Ref.
120		2	H	--	CH_3	CH_3	6.3[b]	40
79	VUF 8973	2	CH_3	--	CH_3	H	6.6 (7.3[b])	37,40
121		2	———	$(CH_2)_2$	———	H	6.6[b]	40
122		2	CH_3	--	CH_3	CH_3	6.3[b]	40
123	VUF 8397	2	H	--	C_6H_5	H	7.0	37
124	VUF 9028	2	H	CH_2	C_6H_5	H	7.8	37
125	VUF 9029	2	H	$(CH_2)_2$	C_6H_5	H	8.0	37
126	VUF 9051	2	CH_3	$(CH_2)_2$	C_6H_5	H	7.8	37
127	VUF 9030	2	H	$(CH_2)_3$	C_6H_5	H	7.6	37
128	VUF 9031	2	H	$(CH_2)_4$	C_6H_5	H	7.7	37
129	VUF 8404	2	H	$(CH_2)_2SCH_2$	C_6H_5	H	7.5 (<5.3[c])	24,37
81	VUF 8328	3	H	--	H	H	8.0	37
130	VUF 9107	3	H	CH_2	C_6H_5	H	8.8	37
131	clobenpropit	3	H	CH_2	C_6H_5-4-Cl	H	9.9	37
132	VUF 9163	3	H	CH_2	c-C_6H_{11}	H	8.8	37
133	VUF 9151	3	H	$(CH_2)_2$	C_6H_5	H	8.8	37
134	iodophenpropit	3	H	$(CH_2)_2$	C_6H_5-4-I	H	9.6	50
135	VUF 9152	3	H	$(CH_2)_3$	C_6H_5	H	8.3	37
136	VUF 4571	3	H	$(CH_2)_4$	C_6H_5	H	8.5	37
137	VUF 8414	3	H	$(CH_2)_2SCH_2$	C_6H_5	H	8.6	37

Table 16 (Continued).

No.	Compound	Structure	pA_2 value[a]	Ref.
83	VUF 8974		5.4	37
138			7.4[b]	52
139			7.5[b]	52

[a] Determined by evaluation of the influence of the compound on electrically evoked, NANC conctractions of guinea pig intestine preparations, as described by Menkveld *et al.*[13]

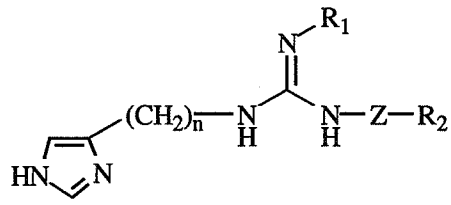
[b] Determined by evaluation of the influence of the compound on K^+-stimulated [^{3}H]-histamine release on rat cortex, as described by Arrang *et al.*[5] Values from patent application.

[c] Determined by evaluation of the influence of the compound on electrically-stimulated [^{3}H]-histamine release on rat cortex, as described by Van der Werf *et al.*[27]

It is interesting to note that Compound **138** might also fit in this series of compounds. Although the isothiourea group is "reversed", a pA_2 value comparable to the ω-phenylalkyl derivatives of imetit is reported in a patent application. The same is true for compound **139**, which may also be regarded as an analogue of imetit. This could be an indication that binding of this series of compounds, depends on the interaction of the nitrogen with the receptor and that the sulphur atom is not important.

The isothiourea group of the imetit analogues has also been replaced by a guanidine group. Although less active, a comparable pattern as with the substituted imetit series (described above) can be observed (see table 17).

Table 17 Histamine H_3 antagonistic activity of some substituted guanidine analogues of imetit.



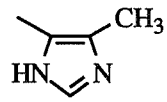
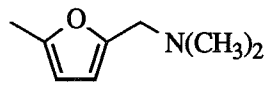
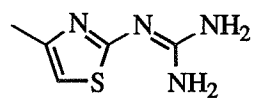
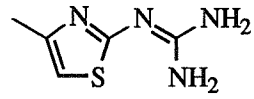
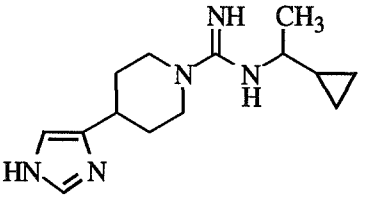
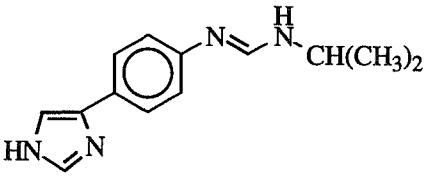
No.	Compound	n	R_1	Z	R_2	pA_2 value	Ref.
77	VUF 83100	2	H	—	H	pD_2=6.3[a] (7.4)[b,c]	37,41
140	VUF 8845	2	NO_2	—	H	inactive[b]	41
141	VUF 9006	2	H	—	phenyl	5.8[a]	37
142	VUF 9007	2	H	$(CH_2)_2$	phenyl	6.3[a]	37
143	VUF 8411	2	H	$(CH_2)_2SCH_2$	phenyl	> 5.3[b]	24
144	SKF 91486	3	H	—	H	7.1[d]	5
145		3	H	CH_2	cyclohexyl	9.2[e] (8.8[f])	53
146		3	$CO_2C(CH_3)_3$	CH_2	cyclohexyl	8.7[e] (7.4[f])	53
147	VUF 8405	3	H	$(CH_2)_2SCH_2$	phenyl	7.9[a]	37
148	impromidine	3	H	$(CH_2)_2SCH_2$		7.2[a]	5
149	VUF 8413	3	H	$(CH_2)_2SCH_2$		6.9[a]	41
150	VUF 8407	3	H	$(CH_2)_2SCH_2$		7.4[a]	41
151	VUF 8406	3	CN	$(CH_2)_2SCH_2$		> 5.3[b]	24
152		4	$CO_2C(CH_3)_3$	—	cyclohexyl	7.3[e] (6.9[f])	53

Table 17 (Continued).

No.	Compound	Structure	pA_2 value	Ref.
153			7.9[d]	48
154	mifentidine		7.0[d]	54

[a] Determined by evaluation of the influence of the compound on electrically evoked, NANC conctractions of guinea pig intestine preparations, as described by Menkveld *et al.*[13]

[b] Determined by evaluation of the influence of the compound on electrically-stimulated [^{3}H]-histamine release on rat cortex, as described by Van der Werf *et al.*[27]

[c] Partial agonist ($\alpha = 0.5$).

[d] Determined by evaluation of the influence of the compound on K^+-stimulated [^{3}H]-histamine release on rat cortex, as described by Arrang *et al.*[5]

[e] Determined by evaluation of the influence of the compound on electrically stimulated [^{3}H]-noradrenaline release on mouse cortex, as described by Schlicker *et al.*[55]

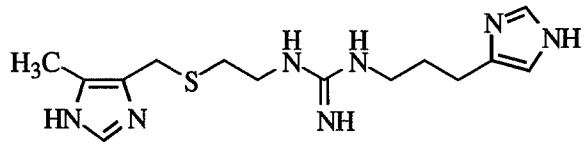
[f] Determined by evaluation of the influence of the compound on electrically evoked, cholinergic conctractions of guinea pig intestine preparations, as described by Vollinga *et al.*[15]

Elongation of the ethylene chain of VUF 83100 (**77**) leads to a reasonably active H_3 antagonist SKF 91486 (**144**). A remarkable hundred-fold increase of H_3 antagonistic activity is observed if a cyclohexyl group, separated by a methylene group is introduced to the guanidine group of SKF 91486 (**144**) (see compound **145**). Esterification of the guanidine group of **145** reduces the antagonistic activity (compound **146**). It is worth noting that this reduction is more drastic on the guinea pig intestine assay than on the mouse cortex assay. The butylene derivative **152** is less active than its propylene analogues, but the compound is still reasonably active. This might explain the H_3 antagonistic properties of mifentidine (**154**) (comparable distance between the imidazole ring and the amidine system).

The propylene chain between the imidazole ring and the amidine system however is optimal for antagonistic activity for this series of guanidine analogues, which was also observed for the isothiourea analogues, described in table 16. Since both series of analogues are likely to interact in a comparable manner with the H_3 receptor, it seems that the nitrogen of the guanidine group, connected directly to the 4(5)-alkylimidazole moiety, is not required for binding.

Derivatives of impromidine as H_3 antagonists

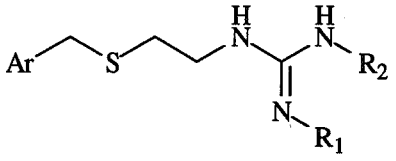
The H_3 antagonistic activity of the H_2 agonist impromidine (**148**)was described already in the first paper on the H_3 receptor.[5]



148, impromidine

The replacement of the propylene chain which separates the unsubstituted imidazole ring and the guanidine group, with an ethylene chain, results in a slight increase of antagonistic activity (see table 18, compound **155**). Methylation of the ethylene chain in a comparable manner as for α-methylhistamine, does not have much effect on the antagonistic activity (enantiomers of sopromidine **156** and **157**). Since no stereoselectivity can be observed between these compounds, an interaction of the unsubstituted imidazole ring in the same manner as for agonists, must be ruled out.

Table 18 Impromidine analogues



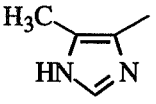
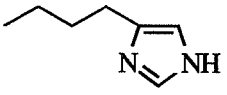
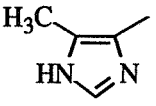
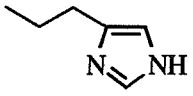
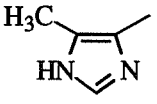
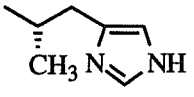
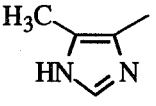
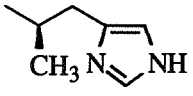
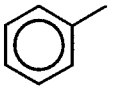
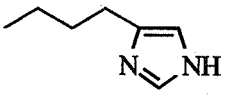
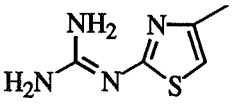
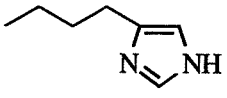
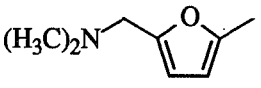
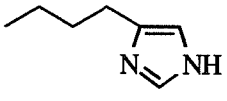
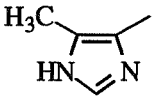
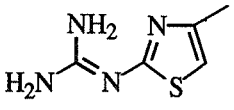
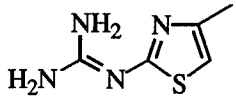
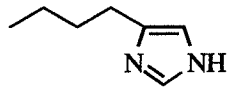
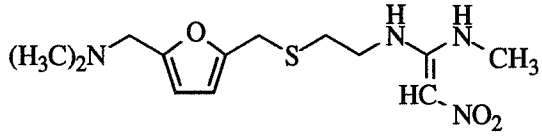
No.	Compound	Ar	R_1	R_2	pA_2 value[a]	Ref.
148	impromidine		H		7.2	5
155			H		7.7	26
156	(S)-sopromidine		H		7.4	35
157	(R)-sopromidine		H		7.2	35
147	VUF 8405		H		7.9[b]	37
150	VUF 8407		H		7.4[b]	41
149	VUF 8413		H		6.9[b]	41
158	cimetidine		CN	CH_3	4.5	5
159	tiotidine		CN	CH_3	4.8	41

Table 18 Impromidine analogues

No.	Compound	Ar	R_1	R_2	pA_2 value[a]	Ref.
151	VUF 8406		CN		> 5.3[c]	24
159	ranitidine				4.3	41

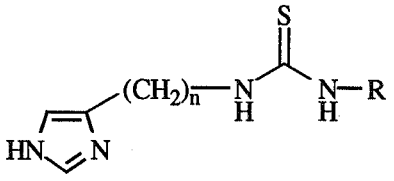
[a] Determined by evaluation of the influence of the compound on K^+-stimulated [^{3}H]-histamine release on rat cortex, as described by Arrang *et al.*[5]

[b] Determined by evaluation of the influence of the compound on electrically evoked, NANC conctractions of guinea pig intestine preparations, as described by Menkveld *et al.*[13]

[c] Determined by evaluation of the influence of the compound on electrically-stimulated [^{3}H]-histamine release on rat cortex, as described by Van der Werf *et al.*[27]

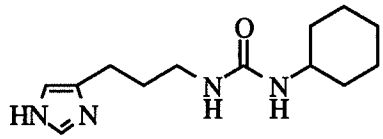
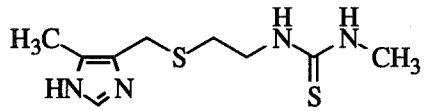
Replacement of the 5-methyl-imidazole ring of impromidine (**148**) is allowed. For instance VUF 8405 (**147**), in which the 5-methyl-imidazole ring is replaced by a phenyl group, is an even more potent H_3 antagonist than impromidine (**148**). This indicates that the 5-methyl-imidazole ring is not essential for the antagonistic activity of impromidine. This is confirmed by the observation that cimetidine (**158**), is almost inactive. Although the introduction of a cyano group to the guanidine, as with cimetidine, might have a negative effect on the antagonistic activity of the compound, it must be noted that VUF 8406 (**151**), with a 4(5)-propyl-imidazole moiety, has been found to be more active. This means that the antagonistic activity of impromidine depends on the unsubstituted imidazole ring and the guanidine system, separated by an alkyl spacer (as described in table 17) and not on the 5-methyl-imidazole part on the other side of the guanidine group.

Table 19 Histamine H_3 antagonistic activity of several analogues of burimamide.



No.	Compound	n	R	pA_2 value[a]	Ref.
161	VUF 4577	2	CH_3	5.5	56
162	VUF 4578	2	C_2H_5	5.3	56
163	VUF 4579	2	C_3H_7	5.4	56
164	VUF 4580	2	$CH(CH_3)_2$	4.8	56
165	VUF 4581	2	c-C_6H_{11}	5.9	56
166	VUF 4582	2	C_6H_5	5.2	56
167	VUF 4583	2	$CH_2C_6H_5$	5.8	56
168	VUF 4584	2	$(CH_2)_2C_6H_5$	5.9	56
169	norburimamide	3	CH_3	6.1 (6.4[b])	15,54,56
170	VUF 4631	3	C_2H_5	7.1	56
171	VUF 4632	3	C_3H_7	7.0	56
172	VUF 4633	3	$CH(CH_3)_2$	7.1	56
173	VUF 4634	3	c-C_6H_{11}	6.9	56
174	VUF 4635	3	C_6H_5	6.9	56
175	VUF 4636	3	$CH_2C_6H_5$	6.7	56
176	VUF 4637	3	$(CH_2)_2C_6H_5$	6.7	56
177	burimamide	4	CH_3	7.1 (7.2[b])	5,15,56
178	VUF 4681	4	C_2H_5	7.4	56
179	VUF 4682	4	C_3H_7	7.3	56
180	VUF 4683	4	$CH(CH_3)_2$	7.5	56
181	VUF 4684	4	c-C_6H_{11}	7.1	56
182	VUF 4685	4	C_6H_5	7.6	56
183	VUF 4686	4	$CH_2C_6H_5$	6.8	56
184	VUF 4687	4	$(CH_2)_2C_6H_5$	7.0	56

Table 19 (Continued).

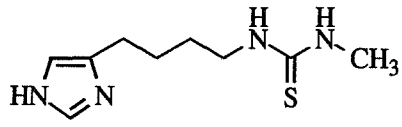
No.	Compound	n	R	pA_2 value[a]	Ref.
185	VUF 4613	5	CH_3	8.0	56
186	VUF 4614	5	C_2H_5	8.0	56
187	VUF 4615	5	C_3H_7	7.7	56
188	VUF 4616	5	$CH(CH_3)_2$	7.7	56
189	VUF 4617	5	c-C_6H_{11}	7.5	56
190	VUF 4618	5	C_6H_5	7.6	56
191	VUF 4619	5	$CH_2C_6H_5$	7.7	56
192	VUF 4742	5	$CH_2C_6H_5$-(4)-Cl	8.1	56
193	VUF 4620	5	$(CH_2)_2C_6H_5$	7.5	56
194	VUF 4740	6	CH_3	7.9	56
195	VUF 4741	6	C_6H_5	8.0	56
196				7.3	53
197	metiamide			5.6[b]	5

[a] Determined by evaluation of the influence of the compound on electrically evoked, cholinergic conctractions of guinea pig intestine preparations, as described by Vollinga *et al.*[15]

[b] Determined by evaluation of the influence of the compound on K^+-stimulated [^{3}H]-histamine release on rat cortex, as described by Arrang *et al.*[5]

Derivatives of burimamide as H_3 antagonists

The good antagonistic activity of the H_2 antagonist burimamide (**177**) for the H_3 receptor led to the development of a large series of burimamide analogues (see table 19 and Chapter 8 for more details).



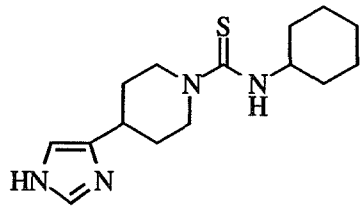
177, burimamide

In this series, the length of the alkyl chain, separating the imidazole ring from the thiourea group, was varied from an ethylene chain to a hexylene chain. Elongation of the alkyl chain clearly results in an increase of the H_3 antagonistic activity. Besides the activity, the selectivity also increased (see Chapter 8).[56] The pentylene- and hexylene analogues of burimamide are about ten times more potent than burimamide (**177**) itself.

The N-thiourea substituents however do not have much influence on the activity. This could mean that this series of antagonists bind in a non-lipophilic environment to the receptor, different from e.g. the amidine analogues, described in table 16 and 17. Also in this series, methylation of the imidazole ring seems to be disastrous, since metiamide (**197**) is only a very weak antagonist. The replacement of the thiourea group of VUF 4634 (**173**) with an urea group (compound **196**) is obviously allowed.

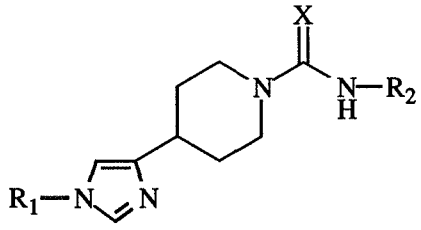
Thioperamide derivatives

The first potent and selective antagonist for the H_3 receptor was thioperamide (**198**), which can be regarded as a rigid analogue of norburimamide (**169**) and VUF 4634 (**173**).



198, thioperamide

Table 20 Histamine H_3 antagonistic activity of some analogues of thioperamide.



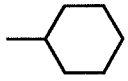
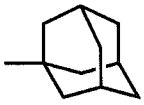
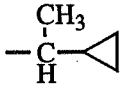
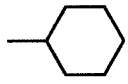
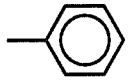
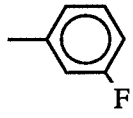
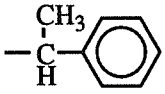
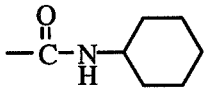
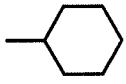
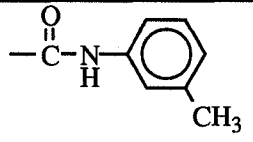
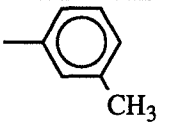
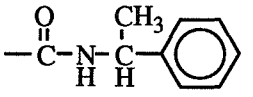
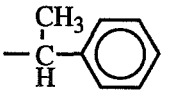
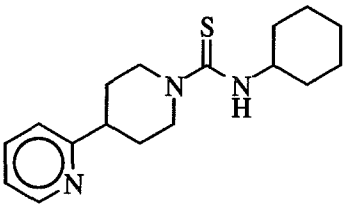
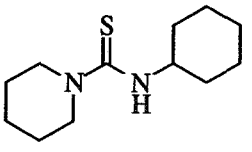
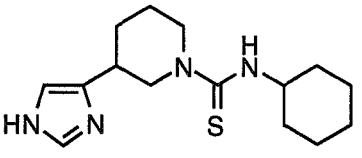
No.	R_1	X	R_2	pA_2 value[a]	Ref.
198	H	S		8.9[b]	5,15
199	H	S		7.5	48
200	H	NH		7.9	48
201	H	O		7.4	48
202	H	O		6.8	48
203	H	O		7.6	48
204	H	O		8.0	48
205		O		7.6	48

Table 20 (Continued).

No.	R_1	X	R_2	pA_2 value[a]	Ref.
206		O		7.1	48
207		O		7.7	48
208				4.9	26
209				< 5.0	57
210				7.4	48

[a] Determined by evaluation of the influence of the compound on K^+-stimulated [^{3}H]-histamine release on rat cortex, as described by Arrang *et al.*[5]

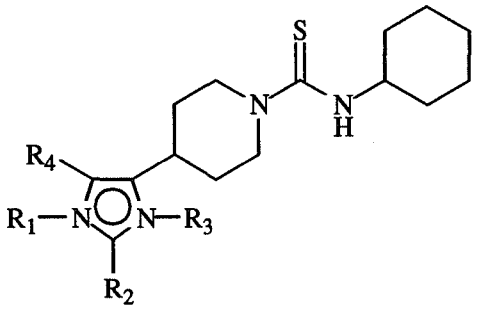
[b] Same value determined by evaluation of the influence of the compound on electrically evoked, cholinergic conctractions of guinea pig intestine preparations, as described by Vollinga *et al.*[15]

According to patent literature,[48] replacement of the cyclohexyl ring with the bulky adamantane group results in a large decrease of antagonistic activity (compound **199**). Moreover the replacement of the thiourea group of thioperamide by an urea group, results in a less active compound (**201**). This is puzzling, since the substitution of a thiourea group of norburimamide (**169**), by an urea group (see table 19, compound **196**), did not result in a decrease of activity. Apparently norburimamide (**169**) and its rigid analogue thioperamide (**198**), bind in a distinct manner to the

receptor. Substituents on the τ-position of the imidazole ring of some urea-containing thioperamide analogues, are allowed (e.g. compound **201** compared to compound **205**).

More substitutions on the imidazole ring of thioperamide have been performed (see table 21). For thioperamide (**198**), methylation of the N^{τ}-position leads to a drastic decrease of activity (N^{τ}-methylthioperamide, **211**), but methylation of the N^{π}-position results in a completely inactive compound **215**.

Table 21 Histamine H_3 antagonistic activity of some substituted thioperamide analogues.



No.	Compound	R_1	R_2	R_3	R_4	pA_2 value[a]	pK_i value	Ref.
198	thioperamide	H	H		H	8.9	8.5[b] (7.4[c])	58,59
211	N^{τ}-methylthioperamide	CH_3	H		H	6.8	6.8[b], 7.2[d] (5.9[c])	58-60
212	N^{τ}-benzylthioperamide	$CH_2C_6H_5$	H		H	N.D.	< 6.0[d]	60
213	2-methylthioperamide	H	CH_3	-	H	N.A.	4.5[b] (4.9[c])	58,59
214	2-aminothioperamide	H	NH_2	-	H	N.A.	< 4.0[b,c]	58,59
215	N^{π}-methylthioperamide	-	H	CH_3	H	N.A.	5.6[b] (5.0[c])	58,59
216	5-methylthioperamide	H	H		CH_3	8.4	6.8[b] (4.6[c])	58,59
217	5-aminothioperamide	H	H		NH_2	N.A.	< 4.0[b,c]	58,59

N.D. = Not Determined; N.A. = Not Active

[a] Determined by evaluation of the influence of the compound on electrically evoked, cholinergic conctractions of guinea pig intestine preparations, as described by Vollinga *et al.*[15]

[b] Affinity value, determined on rat cortex, measuring the displacement of [^{3}H] N^{α}-methylhistamine, as described by Bordi *et al.*[59]

[c] Affinity value, determined on rat cortex, measuring the displacement of [^{3}H] histamine, as described by Bordi *et al.*[59]

[d] Affinity value, determined on rat cortex, measuring the displacement of [^{3}H] N^{α}-methylhistamine, as described by Amin Khan *et al.*[60]

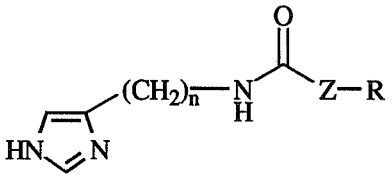
Whereas substituents on the 2-position are not allowed (2-methylthioperamide, **213**), 5-methylthioperamide (**216**) has been reported to be still a potent antagonist.[58] There is however a remarkable discrepancy between the antagonistic activity of 5-methylthioperamide (**216**) as determined on the guinea pig intestine assay and between the much lower affinity of this compound determined on a rat cortex binding assay.

Miscellaneous ω-functionalized 4(5)-alkyl imidazoles

Amides

The observation that the most simple N^{α}-acylated derivative of histamine, N^{α}-acetylhistamine (**218**), displayed weak H_3 antagonistic activity, led to the development of a series of acylated analogues of histamine (see table 22). The introduction of a lipophilic group (e.g. a phenyl- or cycloalkyl group), to the amide group, separated by an alkyl chain results in good antagonists.

Table 22 Histamine H_3 antagonistic activity of several N^{α}-acylated histamine analogues.



No.	n	Z	R	pA_2 value[a]	Ref.
218	2	–	methyl	5.9	61
219	2	CH_2	phenyl	6.0	45
220	2	$(CH_2)_2$	phenyl	6.2	45
221	2	CH_2O	phenyl	6.1	45
222	2	CH_2S	$CH(phenyl)_2$	6.0	45
223	2	$(CH_2)_2$	$CH(phenyl)_2$	6.0	45
224	2	$(CH_2)_3$	phenyl	7.1	45
225	2	CH_2SCH_2	phenyl	6.2	45
226	2	$(CH_2)_3$	cyclohexyl	7.3	45

Table 22. (Continued).

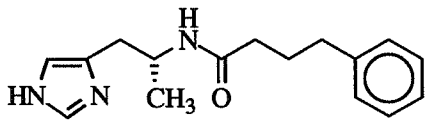
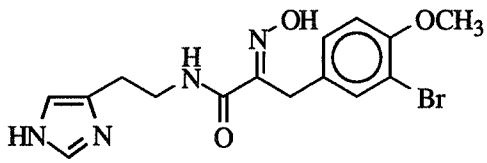
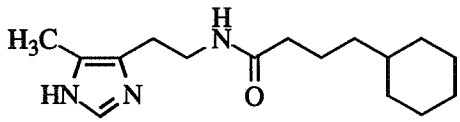
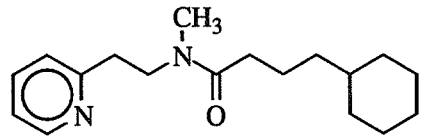
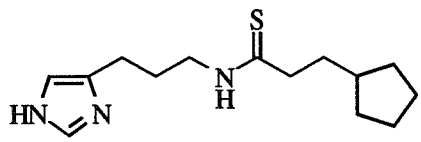
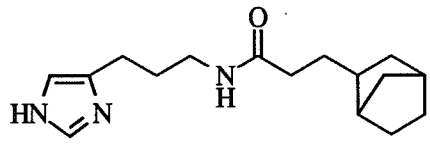
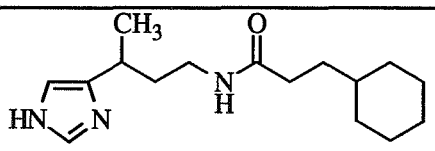
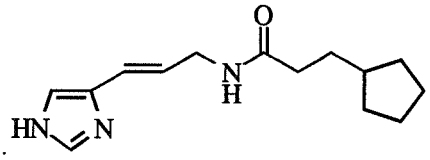
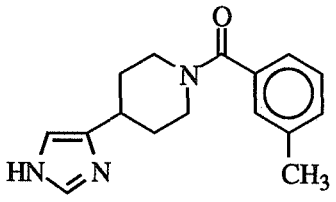
No.	n	Z	R	pA_2 value[a]	Ref.
227	2	$(CH_2)_3$	4(5)-imidazolyl	6.0	45
228	2	$(CH_2)_3$	2-pyridyl	6.0	45
229	2	CH_2SCH_2	2-pyridyl	5.7	45
230	2	$(CH_2)_4$	phenyl	6.7	45
231	3	$(CH_2)_2$	phenyl	7.3	52
232	3	CH_2CHCH_3	phenyl	7.5	52
233	3	$(CH_2)_2$	cyclopentyl	7.2[b] (7.6[c])	53
234	3	$(CH_2)_2$	cyclohexyl	7.3[b] (7.6[c])	53
235	3	$(CH_2)_3$	cyclohexyl	7.2	52
236				6.0	61
237[d]				pK_i = 6.3[e]	62
238				< 5.0	61
239				< 5.0	45
240				7.4[b] (7.6[c])	53
241				7.5[b] (7.7[c])	53

Table 22. (Continued).

No.	Structure	pA_2 value[a]	Ref.
242		8.0[b] (7.8[c])	53
243		7.4	52
244		7.3	48

[a] Determined by evaluation of the influence of the compound on K^+-stimulated [^{3}H]-histamine release on rat cortex, as described by Arrang *et al.*[5]

[b] Determined by evaluation of the influence of the compound on the electrically stimulated [^{3}H]-noradrenaline release on mouse cortex, as described by Schlicker *et al.*[55]

[c] Affinity value (pK_i), determined on rat cortex, measuring the displacement of [^{3}H] N$^\alpha$-methylhistamine, as described by Kathmann *et al.*[63]

[d] Verongamine.

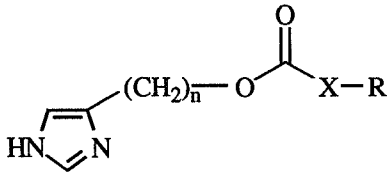
[e] Affinity value, determined on guinea pig brain tissue, measuring the displacement of [^{3}H] N$^\alpha$-methylhistamine, as described by Korte *et al.*[32]

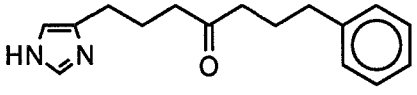
It appears, that the distance of the lipophilic group relatively to the imidazole ring is determining the activity and not the position of the amide group, since **224** and **226** are equipotent as **231** respectively **234**. Apparently the polar group in the side chain is less important for this series of analogues. Also the exchange of the amide group by a thioamide group does not influence the activity (if compound **233** and **240** are compared). Whereas the introduction of a lipophilic group enhances the activity, the introduction of a heterocycle, does not increase the potency (comparing **226** and **227**). Branching of the side chain of these amide analogues leads to an increased activity (see compound **234** and **242**). Methylation in the α-position of **224** however, with the same stereochemistry as (R)-α-methylhistamine (**21**), leads to a

weak antagonist **236**. Methylation or replacement of the imidazole ring is again not allowed.

A remarkable compound is verongamine (**237**), fitting perfectly in this series of compounds. It has been reported as an H_3 antagonist, but only affinity data was provided.[62,64] Verongamine (**237**) is isolated from the marine sponge *Verongula gigantea* and is therefore probably the first discovered endogenous compound with H_3 antagonistic properties.

Table 23 Histamine H_3 antagonistic activity of several carbonylic histaminergic imidazole derivatives.



No.	n	X	R	pA_2 value[a]	Ref.
245	3	$(CH_2)_2$	cyclopentyl	8.5	52
246	3	NH	phenyl	7.9	65
247	3	NH	3-F-phenyl	7.6	65
248	3	NH	4-F-phenyl	7.6	52
249	3	NH	cyclohexyl	7.2	65
250	3	$NHCH_2$	phenyl	8.0	65
251	3	$NHCH_2$	4-F-phenyl	7.6	65
252	3	$NHCH_2$	4-NO_2-phenyl	7.7	52
253	3	$NHCH_2$	cyclopentyl	7.2	65
254	3	$NHCH_2$	cyclohexyl	8.0	65
255	3	$NHCH_2$	2-thiophenyl	7.7	65
256	3	NCH_3CH_2	phenyl	7.7	52
257	3	NHCO	phenyl	7.3	52
258	3	$NH(CH_2)_2$	phenyl	8.0	65
259				7.7	52

[a] Determined by evaluation of the influence of the compound on K^+-stimulated [^{3}H]-histamine release on rat cortex, as described by Arrang *et al.*[5]

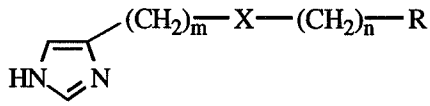
Carbamates, esters and ketones

Other carbonylic histamine derivatives have also been described as H_3 antagonists, like the carbamates presented in table 23. All these compounds are good antagonists, with comparable activities. The carbamate group may even be replaced by a ketone and the activity remains (compound **259**), again diminishing the importance of the polar group for this series of antagonists. Replacement of the carbamate group by a reversed ester group has resulted in a marked increase of activity however (if **253** is compared with **245**).

Ethers

The observation that with the carbonylic antagonists, discussed above, there is no great importance of a functional polar group, seems to be confirmed by a series of ether derivatives (see table 24). In this series, the functional group has been replaced by an oxygen atom and a variety of lipophilic groups with different steric and electronic parameters have been introduced, but all the compounds are comparable good antagonists. A recently reported new radioligand, [^{125}I]-iodoproxyfan (**272**) is described to possess a very high affinity on the rat cortex, but there is a remarkable discrepancy between the reported affinity and the observed antagonistic activity (a hundred times less active) of iodoproxyfan (**272**) on a functional assay.

Table 24 ethers



No.	m	n	R	pA_2 value[a]	Ref.
260	2	0	4-CN-phenyl	8.0	52
261	2	0	4-C_3H_7-phenyl	7.7	52
262	2	0	4-NO_2-phenyl	7.5	52
263	2	0	2-naphthyl	7.0	52
264	3	0	4-CN-phenyl	7.9	52
265	3	0	4-CF_3-phenyl	7.8	52
266	3	1	phenyl	7.7	66
267	3	1	3-F-phenyl	7.9	66
268	3	1	3-I-phenyl	8.2	67
269	3	1	4-Br-phenyl	7.9	66
270	3	1	4-Cl-phenyl	7.7	67
271	3	1	4-F-phenyl	8.0	66
272[b]	3	1	4-I-phenyl	8.3	67
272[b]	3	1	4-[^{125}I]-phenyl	$pK_i = 10.2$[c]	67
273	3	1	cyclohexyl	8.0	66
274	3	1	1-naphthyl	7.7	66
275	3	1	2-naphthyl	7.7	66
276	3	1	2-quinolyl	7.3	66
277	3	1	4-biphenyl	7.2	66
278	3	2	phenyl	7.6	67
279	3	3	phenyl	7.8	67
280	3	3	4-Br-phenyl	7.8	52
281	3	3	4-Cl-phenyl	7.9	52
282	3	3	4-F-phenyl	8.2	52
283	3	3	cyclohexyl	7.7	52

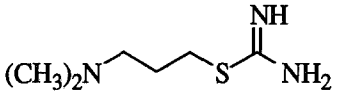
[a] Determined by evaluation of the influence of the compound on K^+-stimulated [^{3}H]-histamine release on rat cortex, as described by Arrang *et al.*[5]

[b] Iodoproxyfan.

[c] Affinity value, determined on rat cortex.

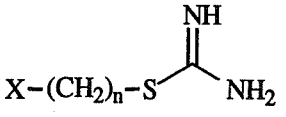
Derivatives of dimaprit as H_3 antagonists

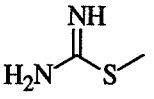
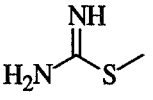
In addition to impromidine (**148**), another H_2 agonist that has been shown to display H_3 antagonistic activity is dimaprit (**284**).



284, dimaprit

Table 25 Histamine H_3 antagonistic activity of some analogues of dimaprit.



No.	Compound	X	n	pA_2 value	pK_i value[a]	Ref.
284	dimaprit	$(CH_3)_2N$	3	6.2[b] (5.5[c])	6.6	54,63
285	nordimaprit	$(CH_3)_2N$	2	5.6[b,d]	6.0	63
286	homodimaprit	$(CH_3)_2N$	4	6.0[b] (5,8[e])	6.4	41,63
99	VUF 8332		2	6.0[f]		41
287	VUF 8333		3	6.0[f]		41

[a] Affinity value, determined on rat cortex, measuring the displacement of [^{3}H] N^{α}-methylhistamine, as described by Kathmann *et al.*[63]

[b] Determined by evaluation of the influence of the compound on electrically stimulated [^{3}H]-noradrenaline release on mouse cortex, as described by Schlicker *et al.*[55]

[c] Determined by evaluation of the influence of the compound on K^+-stimulated [^{3}H]-histamine release on rat cortex, as described by Arrang *et al.*[5]

[d] Reversible non-competitive antagonism.

[e] Determined by evaluation of the influence of the compound on electrically evoked, NANC conctractions of guinea pig intestine preparations, as described by Menkveld *et al.*[13]

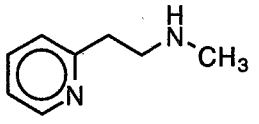
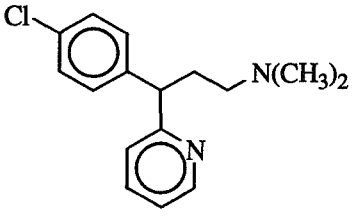
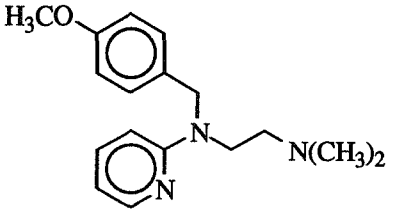
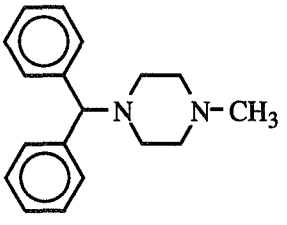
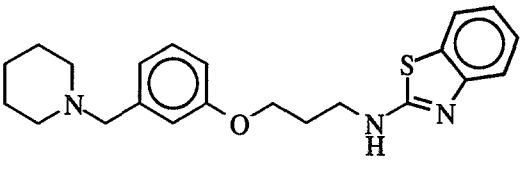
[f] Determined by evaluation of the influence of the compound on electrically-stimulated [^{3}H]-histamine release on rat cortex, as described by Van der Werf *et al.*[27]

A few derivatives of dimaprit (**284**) have been described, which are presented in table 25. Variation in the alkyl chain length, resulted in the compounds nordimaprit (**285**) and homodimaprit (**286**). Whereas shortening the alkyl chain of dimaprit (**284**) results in a decrease of activity, elongation does not effect the activity very much. There is no change in activity when the dimethylamino group of dimaprit is replaced by an isothiourea group (as in VUF 8333, **287**). Shortening the alkyl chain of **287** to two methylene units, does not effect the activity (VUF 8332, **99**). This is not unexpected, since the distance between the amino group and the isothiourea group of homodimaprit (**286**) is comparable to the distance of a nitrogen atom from one of the isothiourea groups of VUF 8332 (**99**) to the other isothiourea group.

Miscellaneous H_3 antagonists

A few other compounds with activity at histaminergic receptors, but lacking an imidazole ring, have been screened for H_3 antagonism. The potent H_1 antagonists chlorpheniramine (**289**) (both enantiomers were tested), mepyramine (**290**) and cyclizine (**291**) do not display any potent antagonistic H_3 activity (see table 26). Moreover the H_2 antagonist zolantidine (**292**) is described to be inactive as an H_3 antagonist as well. Betahistine (**288**) which has been described as a weak agonist on the H_1-and the H_2 receptor, displays a very weak antagonistic activity on the H_3 receptor.

Table 26 miscellaneous

No.	Compound	Structure	pA_2 value[a]	Ref.
288	betahistine		5.2	68
289	chlor-pheniramine		< 7.2[b]	5
290	mepyramine		< 5.5	54
291	cyclizine		< 6.2	5
292	zolantidine		< 5.0	54

[a] Determined by evaluation of the influence of the compound on K^+-stimulated [^{3}H]-histamine release on rat cortex, as described by Arrang *et al.*[5]

[b] Both enantiomers; pA_2 value < 7.2.

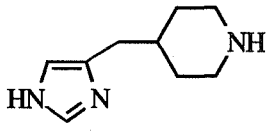
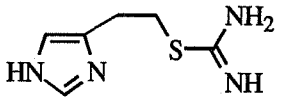
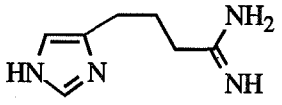
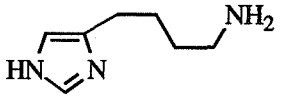
Conclusions

H_3 Agonists

When looking at the structures of all the potent histamine H_3 agonists, that have been developed over the last decade, it is clear that the 4(5)-substituted imidazole ring is essential for activity on the H_3 receptor. Whereas additional substituents on the imidazole lead to strongly reduced activity, replacement of the imidazole by other heterocycles, mostly results in compounds with no H_3 activity at all.
There seems to be more freedom in choice of the side chain. A large series of potent derivatives of histamine has been prepared, based on the introduction of substituents on the aminoethylene side chain connected to the 4(5)-position of the imidazole ring (see table 4-7).

The discovery of the potent agonistic activity of imetit (**75**) however, indicates that the amino group can be substituted by other basic functionality's. It was therefore assumed that an H_3 agonist consisted of an imidazole ring and a basic function, separated by an ethylene chain.
The reported potency of SKF 91606 (**76**) proved that the sulphur atom of the isothiourea group is not required. This suggests that for H_3 agonism, the imidazole ring and a basic function may therefore be separated by a longer alkyl chain than the ethylene chain. This conclusion is confirmed by the activity of the new H_3 agonist immepip (**88**) (see Chapter 6), since the amino group in the structure of immepip is separated from the imidazole ring by a butylene chain.

Table 27. Activity of some potent H_3 agonists, not derived from histamine, as determined on guinea pig jejunum.

No.	Compound	Structure	pD_2 value[a]
88	immepip		8.0
75	imetit		8.1
76	SKF 91606		9.0
110	VUF 4701		$pA_2 = 7.7$

[a] Determined by evaluation of the influence of the compound on electrically evoked, cholinergic conctractions of guinea pig intestine preparations, as described by Vollinga *et al.*[15]

All the potent H_3 agonists reported sofar, comprise a basic function with a cationic nitrogen group. Therefore, it might be assumed that activation of the histamine H_3 receptor by these derivatives, requires the imidazole ring and a cationic nitrogen, at a certain distance in space, relatively to this heterocycle. Based on this assumption it seems that there are two main classes of H_3 agonists:

- the direct histamine analogues where a cationic nitrogen is separated from the imidazole ring by an ethylene chain (see table 4-7) and
- the imetit and immepip analogues, where a cationic nitrogen is separated from the imidazole ring by an alkyl spacer with a chain length, comparable to a butylene chain.

This means that a general structure of a good H_3 agonist at physiological pH, can be represented as:

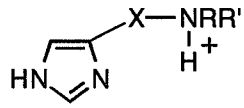


Figure 1. General structure of an H_3 agonist.
Chain length of X, comparable to ethylene or butylene chain.

According to the general structure of an H_3 agonist, as presented in figure 1, VUF 4701 (**110**), which can be seen as a flexible analogue of immepip, should be an agonist. This compound however, is a potent H_3 antagonist on the guinea pig intestine, instead (see Chapter 7). We are currently investigating the activity of VUF 4701 on mouse cortex, measuring [^{3}H]-noradrenaline release.[55] Preliminary results indicate that VUF 4701 is a potent, but partial agonist on this *in vitro* assay.[69] Because VUF 4701 did not show any agonistic activity on the guinea pig jejunum test system (see Chapter 3), this can be an indication for the existence of receptor subtypes; further studies are required however for confirmation.

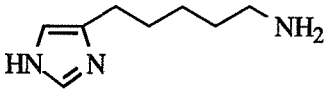
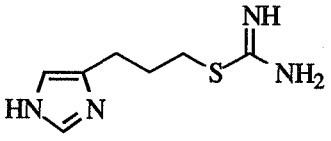
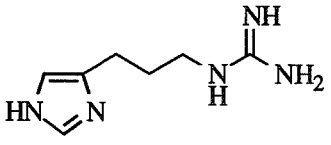
H_3 Antagonists

Also for the H_3 antagonists it is clear that the 4(5)-substituted imidazole ring is essential. There are however many possibility's for variation of the side chain.
Most reported H_3 antagonist may be classified into four distinct groups:

1) the elongated imetit- and histamine analogues,
2) the burimamide analogues,
3) the thioperamide analogues and
4) other ω-functionalized 4(5)-alkyl imidazoles, e.g. the amide-, carbamate-, ester-, and ketone derivatives.

1) The elongated imetit- and histamine analogues, where a cationic nitrogen is separated from the imidazole ring by a pentylene chain are potent antagonists on the guinea pig intestine (see table 28). It might be possible that these derivatives have a comparable binding behaviour and bind to the same sites in the receptor.

Table 28. Comparison of some H_3 antagonists with a cationic nitrogen, located at a comparable distance through bonds, from the imidazole ring.

No.	Compound	Structure	pA_2 value
111	impentamine		8.4[a]
81	VUF 8328		8.0[b]
144	SKF 91486		7.1[c]

[a] Determined by evaluation of the influence of the compound on electrically evoked, cholinergic conctractions of guinea pig intestine preparations, as described by Vollinga *et al.*[15]

[b] Determined by evaluation of the influence of the compound on electrically evoked, NANC conctractions of guinea pig intestine preparations, as described by Menkveld *et al.*[13]

[c] Determined by evaluation of the influence of the compound on K^+-stimulated [^{3}H]-histamine release on rat cortex, as described by Arrang *et al.*[5]

Substitution of the elongated imetit analogue VUF 8328 (**81**) with a lipophilic aralkyl moiety led to very potent antagonists like clobenpropit (**131**). Therefore, we are currently investigating the effect of introduction of these type of substituents on the amino group of impentamine.

2) Another group of H_3 antagonists, probably binding in a distinct manner, are the burimamide analogues (see table 19 and Chapter 8), with a non-protonated thiourea group (at physiological pH). In contradiction to the elongated imetit analogues, the introduction of a lipophilic residue does not lead to a strong increase of antagonistic potency. Variation of the chain length of the alkyl spacer of these burimamide analogues however, has a large influence on the H_3 antagonistic activity, with the H_3 selective pentylene and the hexylene analogues being about ten times more potent than burimamide. Because there is no large influence of the *N*-substituents of the burimamide analogues on the pA_2 value, an interaction with the H_3 receptor via hydrogen bonding seems likely.

3) Thioperamide (**198**) can be regarded as a rigid analogue of norburimamide (**169**). However thioperamide seems to exhibit a different binding behaviour towards the H_3 receptor than the more flexible norburimamide derivatives. For example VUF 4634 (**173**) is about hundred times less potent than thioperamide (see table 19 and chapter 8). Moreover the replacement of the thiourea group of thioperamide by an urea group, led to a strong decrease in H_3 antagonistic activity and replacement of the thiourea group of VUF 4634, did not. Therefore it seems that analogues of burimamide and analogues of thioperamide, although having some structural features in common, do not necessarily interact in a comparable manner with the H_3 receptor.

4) For a fourth distinct group of potent H_3 antagonists, a functional group located at a certain distance in space from the imidazole ring, seems to be less important (see table 22-24). Especially, because of the high potency of the ether analogues presented in table 24, it appears that a lipophilic moiety, separated by an alkyl spacer from the imidazole ring is sufficient for H_3 antagonistic activity.

It must be stressed that it is dangerous to draw solid conclusions about structure activity relationships between H_3 ligands and the H_3 receptor, if the compounds are not tested with the same biological assay. Several discrepancy's in activity of ligands tested on different H_3 *in vitro* assays have been reported in the mean time.

Chapter 3

A Simple and Rapid *In Vitro* Test System for the Screening of Histamine H_3 Ligands

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based on publication :
Methods and Findings in Experimental and Clinical Pharmacology,
14 (10), 747-751 (1992).

Abstract

A simple and rapid functional test system for the screening of histamine H_3 ligands is described. It is based on the inhibitory effect of histamine H_3 agonists on the electrically-evoked contractile response of isolated guinea pig intestine. Whole jejunum segments are continuously stimulated maximally (15-20 V) by electrical pulses with a frequency of 0.1 Hz and a duration of 0.5 msec. The resulting twitches are recorded isotonically (1.0 g) and can be completely abolished by atropine (0.1 μM).

Introduction

The histamine H_3 receptor was first identified by Arrang *et al.*[5] as a presynaptic autoreceptor, regulating the release and synthesis of histamine in nerve endings in the rat cerebral cortex. Until now only a few selective ligands for this receptor have been prepared. The most widely accepted H_3-selective ligands are (R)-α-Methylhistamine as an H_3 agonist and thioperamide as an H_3 antagonist, both able to cross the 'blood brain barrier'.[6]

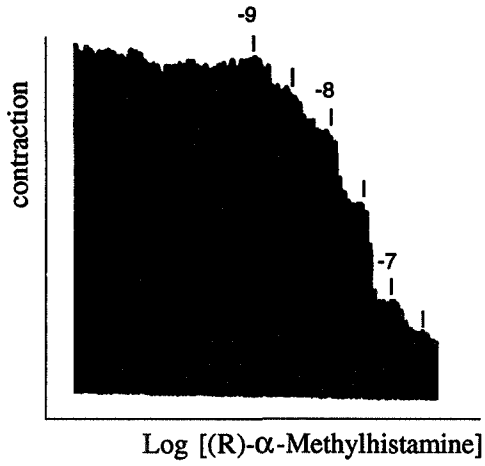
(R)-α-Methylhistamine and thioperamide have been characterized as selective H_3 ligands by an *in vitro* assay based on the release of [^{3}H]-histamine from slices of rat cerebral cortex preloaded with [^{3}H]-histidine.[6] The release of [^{3}H]-histamine can be induced by depolarization with K^+,[5] but also an assay based on superfusion has been described in which [^{3}H]-histamine release is induced by electrical stimulation.[24,27]
Both assays can be used as a test system, but serious drawbacks, make them less attractive for the screening of compounds on H_3 activity. With the K^+-induced-depolarization method, only about 50 % inhibition is found and with the electrically-induced assay, based on superfusion, the response seems to be frequency-dependent, probably due to a frequency dependent receptor reserve.[70,71] Moreover, both procedures are rather complex and laborious. In order to study the structure-activity relationship (SAR) of a series of compounds on H_3 activity, but also for other screening purposes, there is a need for a simple, rapid and reproducible test system.[17]

It is now known that the histamine H_3 receptor also plays a regulatory role in the release of other neurotransmitters (heteroreceptor). For instance the inhibition of noradrenaline-[8] and serotonine-release[7] in the rat brain cortex has been described. The role of the H_3 receptor in the periphery has been established as well, since activation of this receptor inhibits the release of acetylcholine and other neurotransmitters of the non-adrenergic non-cholinergic (NANC) type, in for instance the guinea-pig intestine[72] and guinea-pig airways.[11,12]

The inhibitory action of histamine on electrically evoked, atropine-resistant contractions, of plexus-containing longitudinal muscle preparations from the guinea-pig ileum, has first been demonstrated by Ambache *et al.*[73] The histamine H_2 antagonist burimamide could block this inhibiting effect of histamine, but insensitivity to H_2 agonists[74] made it doubtful that the responsible inhibitory receptor is of the H_2 subtype. In a study by Fjalland *et al.*[75] using whole ileum segments, a distinct difference was demonstrated between the "classical" histamine H_2 receptors in the heart and the histamine-stimulated, contraction-inhibiting receptors on the guinea-pig ileum. The antagonistic effect of burimamide on the inhibitory guinea-pig ileum receptor, was about 25 times higher than that of cimetidine, whereas on the heart cimetidine was found to be 10 to 50 times more potent than burimamide. With the development of the selective H_3 ligands, Trzeciakowski subsequently demonstrated that the observed inhibition of field stimulated contractions of the ileum, was mediated by the H_3 receptor.[76] With this knowledge several papers have been published, describing the existence of H_3 receptors on the guinea-pig small intestine.[13,14,77]

In the present paper we describe a simple assay, based on the functional response of electrically stimulated whole guinea-pig jejunum segments (contractions induced by endogenous acetylcholine-release), which is suitable as an *in vitro* test system for the rapid screening of compounds on histamine H_3 activity. The results are compared with other possible test systems.

(A)



(B)

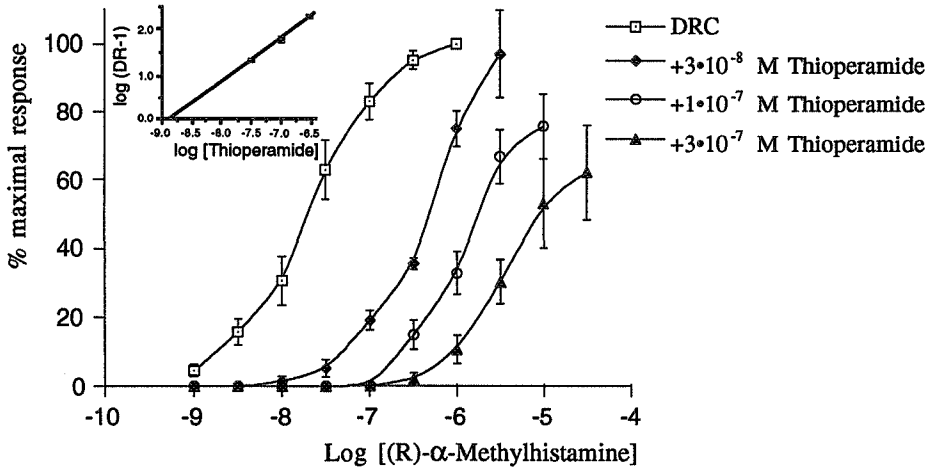


Figure 1 (A) The effect of (R)-α-methylhistamine on the contractile response (twitch) of whole jejunum segments of the guinea pig intestine. (B) Concentration-response curves of (R)-α-methylhistamine during the course of the day, with a rightward parallel shift upon addition of thioperamide. The Schild plot of these results is shown in the inset.

Results

A regular contraction pattern of twitches results from the described stimulation conditions, due to the electrically stimulated release of acetylcholine from the myenteric plexus in the jejunum preparation. These contractions can be completely blocked by atropine (0.1 μM). Applying cumulative concentrations of a histamine H_3 agonist, a concentration dependent inhibition of the twitch response, of the jejunum preparation, is found (Fig. 1A) and a concentration-response curve (CRC) can be constructed (Fig. 1B). A rightward parallel shift of the CRC of (R)-α-Methylhistamine can be observed, after addition of an H_3 antagonist. The maximal relaxation (inhibition of the twitch response) that can be achieved by (R)-α-Methylhistamine, lies between 80 and 100 % of the maximal twitch response (see Fig. 1A). A small decrease in this maximal response of the preparation, upon addition of an H_3 agonist, may be observed after several experiments during the course of the day (see Fig. 1B), possibly due to desensitization. The pD_2 values however remain the same when only CRC's of (R)-α-Methylhistamine are being recorded. About five CRC's can be constructed using one preparation. The first measurement is discarded, because of its sometimes irregular response pattern. In this respect, the addition of a single dose of the H_3 agonist, giving about 90 % of the maximal response, instead of applying cumulative concentrations for the first CRC, is a good option. To prevent activation of the histamine H_1 receptors (also present in the preparation) from interfering with the contraction pattern, pyrilamine or tripolidine can be added (10^{-7} M; no interfering activities at that concentration). Activation of the H_1 receptor results in a rise of the basal tension of the muscle preparation.

It should be noted that there is some confusion in literature about the region of the guinea-pig small intestine that is defined as the ileum. The section between 20-40 cm proximal to the ileocaecal valve is called ileum,[73,76] but also the sections between 5-25 cm,[13] and 10-20 cm.[16] To avoid the anomalous part of the small intestine, we used the section between 20-50 cm proximal to the ileocaecal valve, which we indicate as jejunum according to literature.[72,78]

Table I. Activities of some histamine H_3 ligands, determined with the described test system and compared with other *in vitro* assays

H_3 agonist	H_3 activity parameter	Test system[1]	other H_3 *in vitro* assays A	B	C	D	E	F
(R)-α-methyl-histamine	pD_2	7.8 ± 0.2	7.8	7.6	8.3	7.6	8.4	
N^{α}-methyl-histamine	pD_2	7.7 ± 0.1		7.2	8.4	7.7	7.8	8.7
thioperamide	pA_2 (Schild-slope)	8.9 ± 0.1 (1.0±0.1)	9.0 (1.1±0.1)	7.9	8.5	8.2 (1.0±0.1)	8.9	8.4
burimamide	pA_2 (Schild-slope)	7.1 ± 0.1 (1.0±0.1)	7.5 (1.3±0.1)*			7.0 (0.9±0.1)	7.2	
norburimamide	pA_2 (Schild-slope)	6.4 ± 0.2 (1.0±0.1)	6.4 (1.3±0.1)*				6.1	

[1] Values obtained with our test system represent mean ± SD from 4 different animals in duplo.

* Schild slope significantly different from unity ($p<0.05$; *t* test).

A *In vitro* assay, based on the inhibitory effect of H_3 agonists on electrically evoked, cholinergic conctractions of **guinea pig ileum** preparations, as described by Hew *et al.*[77]

B *In vitro* assay, based on the inhibitory effect of H_3 agonists on electrically evoked, cholinergic conctractions of **guinea pig duodenum** preparations, as described by Coruzzi *et al.*[14]

C *In vitro* assay, based on the inhibitory effect of H_3 agonists on electrically evoked, NANC conctractions of **guinea pig ileum** preparations, as described by Menkveld *et al.*[13]

D *In vitro* assay, based on the inhibitory effect of H_3 agonists on electrically evoked, NANC conctractions of **guinea pig ileum** preparations, as described by Taylor *et al.*[16]

E *In vitro* assay, based on the inhibitory effect of H_3 agonists on K^+-stimulated [^{3}H]-histamine release on **rat cortex**, as described by Arrang *et al.*[5]

F *In vitro* assay, based on the inhibitory effect of H_3 agonists on electrically-stimulated [^{3}H]-histamine release on **rat cortex**, as described by Van der Werf *et al.*[24,27]

Discussion

In this test system, the capacity of a compound to stimulate or block the histamine H_3 receptor, is determined by its ability to inhibit the contractions of a jejunum preparation, which are induced by the release of acetylcholine from myenteric nerve endings in the guinea-pig small intestine, evoked by field stimulation. Such a system has also been described by Fjalland *et al.*,[75] Trzeciakowski[76] and Hew *et al.*[77] on the ileum and by Coruzzi *et al.*[14] on the duodenum. The action of H_3 agonists on non-adrenergic non-cholinergic (NANC) neurotransmitter(s) mediated contractions, has been described by Ambache *et al.*,[73,74] Menkveld *et al.*[13] and Taylor *et al.*[16] The results indicate the presence of H_3 receptors on both cholinergic and non-cholinergic nerves in the myenteric plexus of the small intestine.

With the Ach-mediated as well as with the NANC-mediated response, a concentration dependent inhibition of the contraction-amplitude can be observed, upon addition of an H_3 agonist. The H_3 activities of some selective H_3 ligands, determined by the different *in vitro* assays in literature, are compared in Table I. From these data, it is clear that all these methods can be used for the determination of the H_3 activity of compounds but that the variation between the values obtained by the different assays, is too large for structure-activity relationship (SAR) studies. This means that their is a need for one *standard* test system.

We already mentioned that the laborious rat cortex release assays[6,24,27] are not suitable for this purpose. The assays based on the release of NANC neurotransmitter(s)[13,16,73,74] can be used as an *in vitro* test system for H_3 ligands,[13] but the vulnerable and troublesome preparations (longitudinal muscle strips containing the myenteric plexus, which are difficult to prepare and handle) make this assay less attractive for rapid screening purposes. Another disadvantage may be that it is not known at all, what neurotransmitter(s) evoke(s) the contraction. Of the assays based on the release of acetylcholine, the method described by Coruzzi *et al.*,[14] using segments of the duodenum, is not applicable, because of the described marked desensitization to H_3 agonists (only one CRC for each H_3 agonist can be constructed) and the partial inhibition (50-60 %) of the evoked contraction of the preparation.

When comparing the data obtained from our described test system with the data obtained from the *in vitro* assay described by Hew *et al.*[77] (describing the use of whole segments of the guinea-pig ileum) in Table I, it is clear that there is a great resemblance (although with the ileum preparation, the Schild-slope of Burimamide

and Norburimamide is significantly different from unity). This means that the use of a whole segment of the guinea-pig small intestine (not duodenum) gives reproducible results and is therefore suitable as preparation for our purposes.

In this paper we have described a rapid and reliable *in vitro* test system. Whole jejunum segments are used as preparations, which are easy too prepare and handle and give a quick and reproducible response, using simple electrical stimulation conditions. For these reasons, we think that this assay is most suitable for the screening of compounds on H_3 activity.

Material and methods

Male albino guinea-pigs (350-450 g) were killed by a blow on the head. A portion of the small intestine, 20-50 cm proximal to the ileocaecal valve (jejunum), was removed and placed in Krebs buffer (composition (mM): NaCl 118, KCl 5.6, $MgSO_4$ 1.18, $CaCl_2$ 2.5, NaH_2PO_4 1.28, $NaHCO_3$ 25 and glucose 5.5). Whole jejunum segments (2 cm) were prepared and mounted between two platinum electrodes (4 mm apart) in 20 ml Krebs buffer, continuously gassed with 95% O_2 : 5% CO_2 and maintained at 37°C.

Contractions were recorded isotonically under 1.0 g tension with a Hugo Sachs Hebel -Messvorsatz (TL-2)/HF-modem (Hugo Sachs Elektronik, Hugstetten, Germany) connected to a pen recorder. After equilibration for one hour with washings every 10 min, the muscle segments were stimulated maximally between 15 and 20 Volt and continuously at a frequency of 0.1 Hz and a duration of 0.5 msec, with rectangular-wave electrical pulses, delivered by a Grass Stimulator S-88 (Grass Instruments Co., Quincy, USA). After 30 min of stimulation, cumulative concentration-response curves (half-log increments) of the histamine H_3 agonists were recorded until no change in response was found. H_3 antagonists were added 20 min before generation of concentration-response curves with (R)-α-Methylhistamine as H_3 agonist. Between two succeeding measurements, the preparations were washed three times every 10 minutes, without any stimulation.

The following drugs were used: (R)-α-Methylhistamine dihydrogenmaleate and thioperamide (kindly donated by J.-C. Schwartz, Centre Paul Broca de I'INSERM, Paris), N^{α}-Methylhistamine (Calbiochem-Behring), burimamide (Smith-Kline & French Laboratories) and norburimamide dioxalate (from our own laboratory stock).

The data obtained with the described test system are expressed as mean ± SD Tissue preparations from at least four different animals were used for each compound. Statistical analysis was carried out with the Students' *t*-test. In all tests $p < 0.05$ was considered statistically significant. The potency of an agonist, as an inhibitor of the electrically evoked contractions, was expressed by its pD_2 value. The potency of an antagonist was expressed by its pA_2 value, calculated from the Schild regression analysis where at least three concentrations were used. All other data shown from literature are expressed as pD_2 respectively pA_2 as well, for easy comparison.

Chapter 4

Possible Routes for the Synthesis of 4(5)-Substituted Imidazoles: an Overview

Introduction

When comparing the structures of all the potent histamine H_3 ligands, described in Chapter 2, it is obvious that the 4(5)-substituted-imidazole moiety, is essential for agonistic or antagonistic activity on the H_3 receptor. Since the purpose of this research was the development of new H_3 ligands for SAR- and molecular pharmacological studies, we needed a suitable route for the synthesis of a variety of different 4(5)-substituted imidazoles.

Cyclization Reactions

The classical method of preparing 4(5)-substituted imidazoles derivatives, is based on the use of a linear synthesis route, with a ring closure of the imidazole ring in the final stages.
Some examples of these methods are shown in Figure 1. The most widely used method is known as the Bredereck reaction; route (a).[79-81] For instance 4(5)-hydroxymethyl-imidazole[82] and 4(5)-(2-hydroxyethyl)-imidazole[83] have been prepared by the reaction of the corresponding α-hydroxyketon, with formamidine, respectively formaldehyde in ammonia. Frequently formamide is used for the ring-closure. However, harsh conditions (like high temperatures) are often required for the Bredereck reaction.
Another frequently used method is the Marck-Wald reaction; route (b). This route has been used by Schunack for the synthesis of thioperamide.[84]
A milder ring-closure method has been described more recently by Shih; route (c).[85] Here an *N*-trimethylsilylimine reacts with the anion of tosylmethylisocyanate (reaction temperature; -78°C to room temperature), followed by hydrolysis to obtain the imidazole.

In general and from our own experiences however, imidazole-cyclization reactions are low yielding, time-consuming and give many side products, which are difficult to remove. Also, the reagents for the cyclization reaction are often difficult to obtain. Another large drawback is that this linear synthesis route is rather inflexible, meaning that for most desired structural variations, a totally new route has to be developed. It would be more convenient to use a convergent synthesis route, in which the (intact) imidazole ring is our starting material, to which many different substituents can be coupled directly to the 4(5)-position. We therefore developed a new route for the synthesis of various 4(5)-substituted imidazoles.

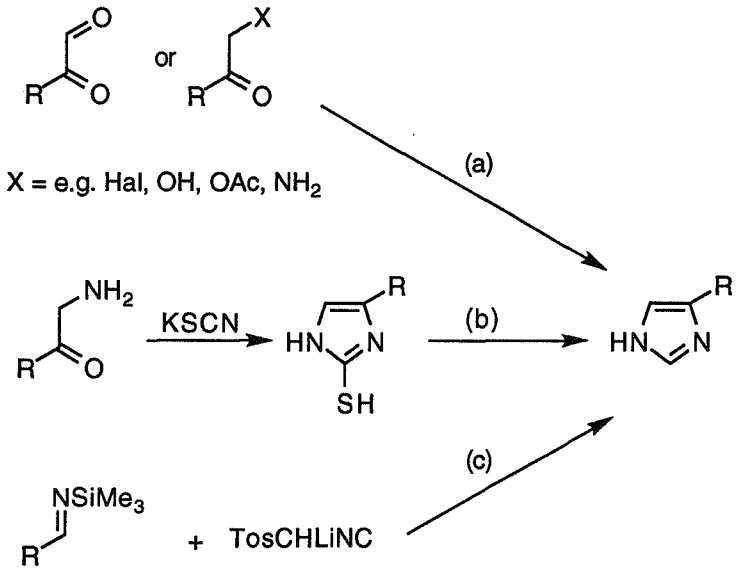


Figure 1 Some classical routes to 4(5)-substituted imidazoles,

Coupling Reactions

Not many preparative routes for the synthesis of 4(5)-substituted imidazoles, using a direct coupling reaction, have been described yet. However several preliminary investigations have been (and are currently) published.

Shirley[86] and Roe[87] demonstrated already more than 30 years ago, that 1-substituted imidazoles can be metallated at the C2-position using *n*-butyllithium and treated subsequently with an electrophile, leading to a 1,2-disubstituted imidazole. About 15 years later it was demonstrated by Tang[88] that it is also possible to lithiate the C5-position of the imidazole ring, if both the 1- and the 2-position are substituted. The addition of carbonyl compounds resulted in the formation of the corresponding alcohols in good yields. Since then, several lithiation procedures for the imidazole ring and substitutions with a variety of electrophiles , have been reported.[89-100]

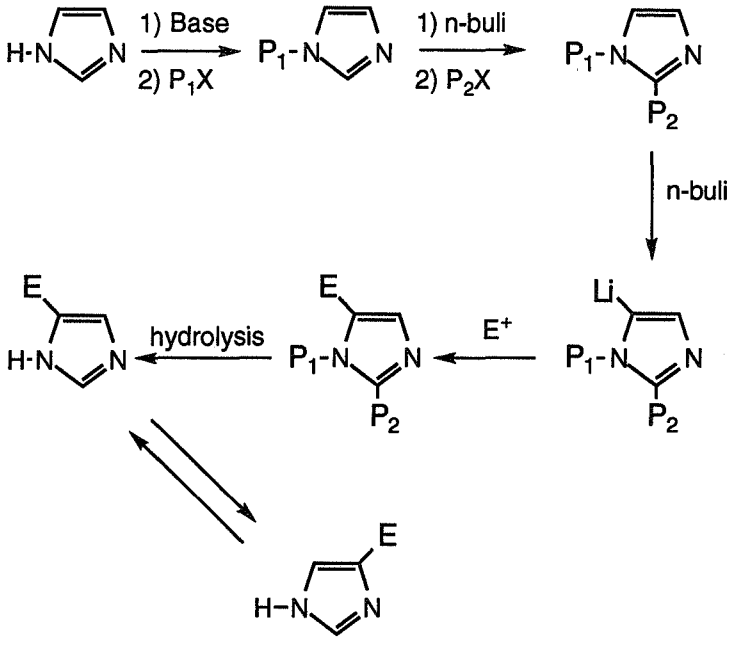
Not only direct metallation reactions, but also metal-halogen exchange reactions with poly-iodinated or poly-brominated 1-protected imidazoles have been described.[89,92,93,101-106] In theory it should be possible to lithiate and functionalize any of the three carbon atom positions of a suitable *N*-protected polyhaloimidazole. Halogen-metal exchange reactions are mostly used for the synthesis of 4,5-asymmetrically-disubstituted imidazoles.[102,103]

Apart from mono-lithiation of the imidazole ring, 2,5-dilithiation is also possible, but attempts to induce a regioselective electrophilic attack at the C-5 position have met with limited success. Only in the case of methyliodide as electrophile, an excess of the 5-substituted product in a reaction mixture of 5-methyl and 2,5-dimethyl derivatives, has been reported.[90]

In the last few years, a few other potential substitution reactions for the synthesis of a variety of 4(5)-substituted imidazoles, have been described, which still need to be investigated further. For instance allylation of a 1,2-diprotected-5-iodoimidazole with a (π-allyl)nickel-complex[107] or the coupling of an alkyliodide to TMS-imidazole using $TiCl_4$ (low yield)[108] has been reported, but also the direct coupling of aromatic halides to the imidazole ring.[109,110]

Development of a Preparative Synthesis Route

We investigated the possibility to use direct C-5 lithiation reactions of a suitable 1,2-diprotected imidazole, for the synthesis of new H_3 ligands. A general scheme of this approach to 4(5)-substituted imidazoles is shown in Scheme I.



Scheme I. General procedure for the preparation of 4(5)-substituted-1H-imidazoles via C-5 lithiation, in which P_1 and P_2 are protecting groups and E is an electrophile.

The synthesis of 4(5)-substituted imidazoles at a preparative scale through metallation at the C5-position requires an efficient protection of both the N1- and C2-positions, in combination with an easy, preferably 'one pot' deprotection step, under conditions that don't affect other functional groups present. Several different protecting groups have been proposed[89-91,93,94,96,111] and treatment of the metallated intermediates with a suitable electrophile resulted in the corresponding substituted imidazoles. However, only few papers describe the actual synthesis of target compounds, using this approach.[88,94,95,98,100,112]

Most of the reported NH protecting groups reported, were not suitable for our purposes. For instance the benzyl group is not inert under the reaction conditions[86-88,90] and the removal of the methoxymethyl group requires severe deprotection conditions.[88,90] Several other groups where found to be too labile for the use as protecting group for the NH function.[90] We selected the *N,N*-dimethylsulfamoyl group as a protecting group for the NH function of the imidazole ring in our procedures, because it can be introduced and removed under simple and relatively mild conditions and is stable under the lithiation conditions, directing the lithiation to the *ortho*-position next to the sulphonamide group.[90,96]

For protection of the C-2 position of the 1-(*N,N*-dimethylsulfamoyl)imidazole, the phenylthio group was tried initially , but we found that the deprotection of the C-2 position of the 1,2-diprotected-5-substituted imidazole, was not feasible under any of the conditions described in literature.[88,113] The *tert*-butyldimethylsilyl group (TBDMS) proved to be the most convenient protecting group for our purposes, as it is stable to the metallation conditions and can be easily removed using the same conditions as for the removal of the *N,N*-dimethylsulfamoyl group, thereby allowing a 'one-pot' deprotection step. Incidentally, we were able to use the economically more attractive trimethylsilyl group, depending on the reactivity of the added electrophile (see chapter 6 and 9).

Chapter 5

A New Convenient Route for the Synthesis of 4(5)-(ω-Aminoalkyl)-1*H*-imidazoles

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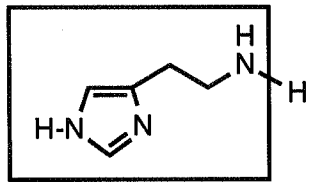
Abstract

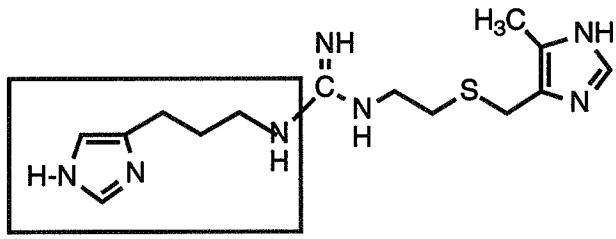
A new route for the synthesis of 4(5)-(ω-aminoalkyl)-1*H*-imidazoles **1** on a preparative scale through C5-lithiation of a 1,2-diprotected imidazole is described. When the described 1,2-diprotected-5-lithio-imidazole is treated with a 1-chloro-ω-iodoalkane **5**, selective substitution of the iodo group takes place. The chloro group of the resultant 1,2-diprotected-5-(ω-chloroalkyl)imidazole can be easily converted into an amino group, but obviously other functional groups can be introduced as well. Subsequent deprotection of the 5-substituted imidazole affords 4(5)-(ω-aminoalkyl)-1*H*-imidazoles **1**, as precursors for a large range of histaminergic compounds, in good overall yields.

Introduction

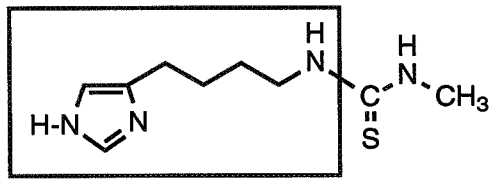
In the search for compounds that selectively bind to histamine receptors and, as a result of this, stimulate (agonists) or block (antagonists) such receptors, several useful drugs have been found. The natural agonist of the histamine receptors (until now three subtypes have been identified; the histamine H_1-,H_2-, and H_3-receptor) is histamine (4(5)-(2-aminoethyl)-1*H*-imidazole). It is therefore not surprising that several histamine ligands, like impromidine and burimamide (Figure 1), possess an 4(5)-(ω-aminoalkyl)-1*H*-imidazole fragment in their structure.



Histamine H_1-, H_2-, H_3- agonist



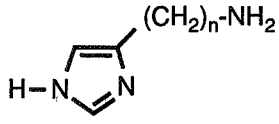
Impromidine H_2- agonist; H_3- antagonist



Burimamide H_2-, H_3- antagonist

Figure 1. Three ligands of histamine receptors, all possessing a 4(5)-(ω-aminoalkyl)-1*H*-imidazole fragment.

A convenient synthesis route for the preparation of 4(5)-(ω-aminoalkyl)-1*H*-imidazoles **1** is therefore of interest.



1

The classical method of preparing this kind of imidazole derivative is by a linear synthesis, with a ring closure (the actual preparation of the imidazole nucleus and the most difficult and low yielding step) in the final and crucial stages.[114-118] The preparation of 4(5)-(3-aminopropyl)-1*H*-imidazole (**1a**) for instance, has been described by chain elongation of 4(5)-(2-chloroethyl)-1*H*-imidazole (prepared by ring closure) with sodium cyanide and subsequent hydrogenation over Raney-Nickel.[114] Also the reaction of 1-bromo-5-phthalimido-2-pentanone (from 5-phthalimido-2-pentanone) with formamidine acetate, followed by hydrolysis, results in the formation of **1a**[116,117] (highest reported overall yield is 14 %).
In general and from our own experiences, these kind of procedures are mostly low yielding and rather time consuming.
Because of the importance of 4(5)-(ω-aminoalkyl)-1*H*-imidazoles **1**, as precursors for a whole range of histaminergic compounds, we have developed an alternative route for a more rapid synthesis of these compounds on a relatively large scale. The tedious ringclosure is avoided within this route, by direct coupling of the alkyl part to the imidazole 4(5)-position through metallation of the ring and subsequent quenching with a bifunctional electrophile.

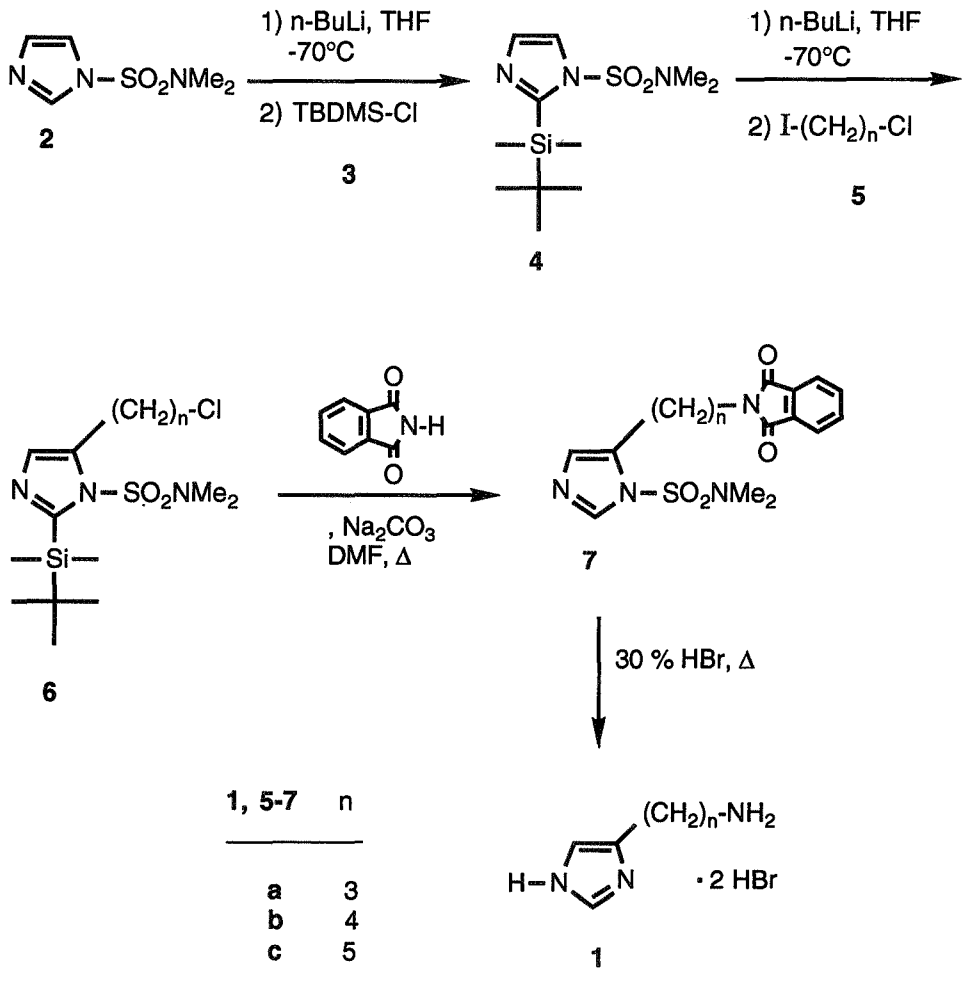
In the present paper a new, rapid procedure for the synthesis of the dihydrobromides of 4(5)-(3-aminopropyl)- (**1a**), 4(5)-(4-aminobutyl)- (**1b**), and 4(5)-(5-aminopentyl)-1*H*-imidazole (**1c**), on a preparative scale (0.15 mol) is described.

Results and Discussion

It has been shown that the imidazole ring can be metallated selectively at either the C2-,[86,87] or at the C5-position[88] after treatment of a 1-protected or a 1,2-diprotected imidazole respectively, with *n*-butyllithium. Several different protecting groups have been proposed[89-91,93,96,97] and treatment of the metallated intermediates with a suitable electrophile yields the corresponding substituted imidazoles. However, only few papers describe the actual synthesis of target compounds, using this approach.[88,94,95,112]

We selected the *N,N*-dimethylsulfamoyl group (Me_2NSO_2-) as a protecting group for the NH function of the imidazole in our procedure, because of its ease of introduction and removal under simple and relatively mild conditions.[90,96] For the C2 protection of 1-(*N,N*-dimethylsulfamoyl)imidazole **2**, the phenylthio group was used as a protecting group initially , but the deprotection of the C2 position of the 1,2-diprotected-5-substituted imidazole, was not feasible under any of the conditions described in literature.[88,113] The *tert*-butyldimethylsilyl group (TBDMS) proved to be the most convenient protecting group in our synthesis, as it is resistant to the metallation conditions and can be easily removed under the same conditions as used for the removal of the *N,N*-dimethylsulfamoyl group, thereby allowing a 'one-pot' deprotection step.

The intermediate 2-(*tert*-butyldimethylsilyl)-1-(*N,N*-dimethylsulfamoyl)imidazole (**4**) can be isolated,[91] but it is more convenient to generate this compound *in situ* and to lithiate at C5 directly by the addition of a second equivalent of *n*-butyllithium. Preliminary experiments indicated that the 1,2-diprotected-5-lithioimidazole can be readily alkylated with *n*-alkyl iodides, but *not* with other *n*-alkyl halides. This selectivity allowed us to use 1-chloro-ω-iodoalkanes **5** as bifunctional electrophiles. With this method we can introduce an alkyl chain with a variable chain length and a ω-chloro group, which can be readily transformed into other groups. We converted the chloro group into an amino group via the *Gabriel synthesis*.[119] This has the advantage that deprotection of the imidazole and hydrolysis of the phthalimide, can be realized in one step, avoiding a separate deprotection step (actually, ^{1}H NMR indicates that the 2-*tert*-butyldimethylsilyl group is already lost under the conditions used for the formation of the phthalimide derivative).



Scheme I. Reaction scheme for the preparation of 4(5)-(ω-aminoalkyl)-1*H*-imidazoles.

The overall yields of the 4(5)-(ω-aminoalkyl)-1*H*-imidazoles **1**, using the described procedure (Scheme I), seem to increase with increasing chain-length (**1a** 26%, **1b** 35% and **1c** 51%). An explanation for this observation could be the relative ease of HI-elimination of the 1-chloro-ω-iodoalkanes (**5**), with the 5-lithio derivative of **4** reacting as a base, especially for the 1-chloro-3-iodopropane (**5a**).

It can be concluded that our method offers a new, quick and high yielding route to histamine analogues on a preparative scale, avoiding a tedious ringclosure. It is obvious that this procedure is not only applicable for the synthesis of the 4(5)-(ω-aminoalkyl)-1*H*-imidazoles, but also for a whole range of other functionalized 4(5)-alkyl-1*H*-imidazoles.

Experimental Section

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC-200 (200 MHz, FT) spectrometer with tetramethylsilane or sodium 3-(trimethylsilyl)propionate as an internal standard. Mass spectra were recorded on a Finnigan MAT-90. Melting points were measured on a Mettler FP-5 + FP-52 apparatus and are uncorrected. THF was distilled from $LiAlH_4$, DMF was dried by passage through a column packed with Al_2O_3 and acetone was distilled from K_2CO_3.
The 1-chloro-ω-iodoalkanes **5** were prepared by refluxing the corresponding 1,ω-dichloroalkanes with 1 eq. of sodium iodide in acetone and purification of the product by distillation.[120]
1-(*N,N*-Dimethylsulfamoyl)imidazole (**2**) was synthesized by the method described by Chadwick and Ngochindo,[90] with the exception that toluene was used as a solvent instead of benzene.

*2-(tert-Butyldimethylsilyl)-5-(3-chloropropyl)-1-(N,N-dimethylsulfamoyl)-imidazole (**6a**) (general procedure)*

1-(*N,N*-Dimethylsulfamoyl)imidazole (**2**) (26.0 g, 0.15 mol) was dissolved in dry THF (500 ml) under an atmosphere of dry nitrogen and cooled to -70°C. *n*-Butyllithium in hexane (100 ml, 0.16 mol) was added dropwise (temperature should not exceed -65°C). After 15 min, a solution of *tert*-butyldimethylsilyl chloride (**3**) (25.0 g, 0.17 mol) in dry THF (60 ml) was added (10 min) and the solution was stirred at room temperature for 1 h. The mixture was cooled to -70°C again and *n*-butyllithium in hexane (100 ml, 0.16 mol) was added dropwise (temperature should not exceed -65°C). After 0.5 h, a solution of 1-chloro-3-iodopropane (**5a**) (35.0 g, 0.17 mol) in dry THF (50 ml) was added gradually and the mixture was allowed to (slowly) warm to room temperature overnight. The reaction mixture was poured into water (200 ml) and the THF was removed under reduced pressure. The product was extracted with $CHCl_3$ (3 x 150 ml), dried (Na_2SO_4) and concentrated *in vacuo*. 53.8 g Of a viscous orange coloured oil was obtained which was used without further purification.

Analysis of the oil by ^{1}H NMR showed formation of the product **6a** and the 1,2-diprotected imidazole **4** in a ratio of 1.5 : 1.
^{1}H NMR ($CDCl_3$): δ 0.38 (s, 6H, $Si(C\underline{H}_3)_2$), 1.02 (s, 9H, $C(C\underline{H}_3)_3$), 2.17 (m, 2H, $CH_2C\underline{H}_2CH_2$), 2.85 (s, 6H, $N(C\underline{H}_3)_2$), 2.94 (t, 2H, J = 8 Hz, imidazole-5-$C\underline{H}_2$), 3.63 (t, 2H, J = 8 Hz, $C\underline{H}_2Cl$), 6.96 (s, 1H, imidazole-4H) ppm.

*2-(tert-Butyldimethylsilyl)-5-(4-chlorobutyl)-1-(N,N-dimethylsulfamoyl)imidazole (**6b**)*

Employing the same procedure as for the preparation of **6a** using 1-chloro-4-iodobutane (**5b**) (37.0 g, 0.17 mol), 60.9 g of a viscous orange coloured oil was obtained which was used without further purification. Analysis of the product by ^{1}H NMR showed formation of the product **6b** and the 1,2-diprotected imidazole **4** in a ratio of 4 : 1.
^{1}H NMR ($CDCl_3$): δ 0.38 (s, 6H, $Si(C\underline{H}_3)_2$), 1.00 (s, 9H, $C(C\underline{H}_3)_3$), 1.86 (m, 4H, $CH_2(C\underline{H}_2)_2CH_2$), 2.67 (t, 2H, J = 8 Hz, imidazole-5-$C\underline{H}_2$), 2.83 (s, 6H, $N(C\underline{H}_3)_2$), 3.56 (t, 2H, J = 7 Hz, $C\underline{H}_2Cl$), 6.94 (s, 1H, imidazole-4H) ppm.

*2-(tert-Butyldimethylsilyl)-5-(5-chloropentyl)-1-(N,N-dimethylsulfamoyl)imidazole (**6c**)*

Employing the same procedure as for the preparation of **6a**, using 17.5 g (0.1 mol) of 1-(*N,N*-dimethylsulfamoyl)imidazole (**2**) in 400 ml dry THF, 65 ml (0.1 mol) *n*-butyl-lithium, 15 g (0.1 mol) *tert*-butyldimethylsilyl chloride (**3**) and 23.3 g (0.1 mol) 1-chloro-5-iodopentane (**5c**). 37.7 g of a viscous orange coloured oil was obtained. Analysis of the product by ^{1}H NMR showed formation of the product **6c** and the 1,2-diprotected imidazole **4** in a ratio of 9 : 1.
^{1}H NMR ($CDCl_3$): δ 0.38 (s, 6H, $Si(C\underline{H}_3)_2$), 1.00 (s, 9H, $C(C\underline{H}_3)_3$), 1.57 (m, 2H, $(CH_2)_2C\underline{H}_2(CH_2)_2$), 1.80 (m, 4H, $(CH_2C\underline{H}_2)_2CH_2$), 2.73 (t, 2H, J = 8 Hz, imidazole-5-$C\underline{H}_2$), 2.84 (s, 6H, $N(C\underline{H}_3)_2$), 3.55 (t, 2H, J = 7 Hz, $C\underline{H}_2Cl$), 6.93 (s, 1H, imidazole-4H) ppm.

*4(5)-(3-Aminopropyl)-1H-imidazole dihydrobromide (**1a**) (general procedure)*

The crude product **6a** (53.8 g) and phthalimide (22.0 g, 0.15 mol) were dissolved in DMF (500 ml), Na_2CO_3 was added (18.0 g, 0.17 mol) and the mixture was heated at 90°C. After 7 h the mixture was filtrated and the solvent was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (150 ml), washed with H_2O (3 x 150 ml), dried (Na_2SO_4) and concentrated *in vacuo*. The substitution of the chloro atom by a phthalimido group and the removal of the 2-*tert*-butyldimethylsilyl group were complete. The formation of 1-(*N,N*-dimethylsulfamoyl)-5-(3-phthalimidopropyl)imidazole (**7a**) was demonstrated by ^1H NMR.

^1H NMR ($CDCl_3$): δ 2.09 (m, 2H, $CH_2C\underline{H}_2CH_2$), 2.86 (t, 2H, imidazole-5-$C\underline{H}_2$),2.90 (s, 6H, $N(C\underline{H}_3)_2$), 3.80 (t, 2H, $C\underline{H}_2$-phthalimide), 6.94 (s, 1H, imidazole-4H), 7.80 (m, 5H, phthalimide-H + imidazole-2H) ppm.

The crude product was dissolved in 30 % HBr (500 ml) and heated under reflux. After 16 h the mixture was cooled, filtrated and concentrated *in vacuo*. The residue was dissolved in absolute EtOH (300 ml), heated under reflux for 0.5 h and concentrated under reduced pressure. The remaining dark oil was washed (under stirring) with portions (100 ml) of acetone. After several washings, the oil crystallized. After filtration of the grey crystals, 11.2 g (26 % overall) of the product **1a** was obtained. Recrystallization from EtOH/EtOAc gave white crystals.

m_p : (193.8-195.2) °C (the melting point of **1a** as a di-HCl salt is given in ref. [114]).

^1H NMR (D_2O): δ 2.08 (m, 2H, J = 8 Hz, $CH_2C\underline{H}_2CH_2$), 2.88 (t, 2H, J = 8 Hz, imidazole-4(5)-$C\underline{H}_2$), 3.09 (t, 2H, J = 8 Hz, $C\underline{H}_2NH_2$), 7.31 (s, 1H, imidazole-5(4)H), 8.63 (s, 1H, imidazole-2H) ppm.

^{13}C NMR (D_2O): δ 22.5, 27.2, 40.1, 117.2, 133.5, 134.6 ppm.

MS (CI, methanol): m/e 126 (100 %, MH^+), 109 (43 %, MH^+- NH_3^+), 95 (18 %, MH^+- CNH_4), 79 (11 %).

*4(5)-(4-Aminobutyl)-1H-imidazole dihydrobromide (**1b**)*

Employing the same procedure as for the preparation of **1b**, using the crude product **6b** (60.9 g). This time the intermediate 1-(*N,N*-dimethylsulfamoyl)-5-(4-phthalimidobutyl)imidazole (**7b**) was purified further by washing with petroleum ether (40/60), and trituration with *iso*-propanol. The product crystallized as a beige coloured solid (12.5 g, 31 %).

^1H NMR ($CDCl_3$): δ 1.77 (m, 4H, $CH_2(C\underline{H}_2)_2CH_2$), 2.82 (t, 2H, J = 7 Hz, imidazole-5-$C\underline{H}_2$), 2.92 (s, 6H, $N(C\underline{H}_3)_2$), 3.76 (t, 2H, J = 7 Hz, $C\underline{H}_2$-phthalimide), 6.85 (s, 1H, imidazole-4H), 7.80 (m, 5H, phthalimide-H + imidazole-2H) ppm.

After hydrolysis of this material, 6.0 g (13.5 % overall) of **1b** was isolated as a brownish solid. In another experiment using the crude 1-(*N,N*-dimethylsulfamoyl)-5-(4-phthalimidobutyl)imidazole (**7b**), an overall yield of 35 % of the product **1b** was obtained.

^{1}H NMR (D_2O): δ 1.77 (m, 4H, $CH_2(C\underline{H}_2)_2CH_2$), 2.80 (t, 2H, J = 8 Hz, imidazole-4(5)-$C\underline{H}_2$), 3.06 (t, 2H, J = 8 Hz, $C\underline{H}_2NH_2$), 7.28 (s, 1H, imidazole-5(4)H), 8.59 (s, 1H, imidazole-2H) ppm.

^{13}C NMR (D_2O): δ 24.8, 26.3, 27.5, 40.7, 116.9, 134.3, 134.6 ppm.

MS (CI/MeOH): m/e 140 (100 %, MH^+), 123 (70 %, MH^+- NH_3^+), 79 (55 %).

4(5)-(5-Aminopentyl)-1H-imidazole dihydrobromide (1c)

Employing the same procedure as for the preparation of **1a**, using the crude product **6c** (37.7 g). 1-(*N,N*-dimethylsulfamoyl)-5-(5-phthalimidopentyl)imidazole (**7c**) was isolated as a brownish oil.

^{1}H NMR ($CDCl_3$): δ 1.41 (m, 2H, $(CH_2)_2C\underline{H}_2(CH_2)_2$), 1.70 (m, 4H, $(CH_2C\underline{H}_2)_2CH_2$), 2.69 (t, 2H, J = 8 Hz, imidazole-5-$C\underline{H}_2$), 2.85 (s, 6H, $N(C\underline{H}_3)_2$), 3.67 (t, 2H, J = 7 Hz, $C\underline{H}_2$-phthalimide), 6.79 (s, 1H, imidazole-4H), 7.73 (m, 5H, phthalimide-H + imidazole-2H) ppm.

Hydrolysis of this material gave 16.0 g (51 % overall) of product **1c**.

^{1}H NMR (D_2O): δ 1.45 (m, 2H, $(CH_2)_2C\underline{H}_2(CH_2)_2$), 1.72 (m, 4H, $(CH_2C\underline{H}_2)_2CH_2$), 2.76 (t, 2H, J = 7 Hz, imidazole-4(5)-$C\underline{H}_2$), 3.00 (t, 2H, J = 7 Hz, $C\underline{H}_2NH_2$), 7.23 (s, 1H, imidazole-5(4)H), 8.58 (s, 1H, imidazole-2H) ppm.

^{13}C NMR (D_2O): δ 24.3, 25.1, 26.5, 28.0, 41.1, 116.8, 134.2, 135.2 ppm.

MS (CI, methanol): m/e 154 (100 %, MH^+), 137 (25 %, MH^+- NH_3^+), 123 (79 %, MH^+- CNH_4).

Acknowledgement. We thank Dr. B. van Baar for the determination of the MS data.

Chapter 6

A New Potent and Selective Histamine H_3 Receptor Agonist, 4-(1*H*-imidazol-4-yl-methyl)piperidine (Immepip)

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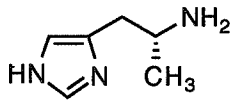
Abstract

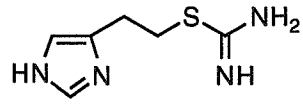
In the search for new selective histamine H_3 receptor ligands, we have identified the new potent histamine H_3 agonist 4-(1*H*-imidazol-4-ylmethyl)piperidine (immepip) (**3**). The agonistic activity and the binding affinity of immepip (**3**) for the H_3 receptor is comparable to the two H_3 agonists (R)-α-methylhistamine (**1**) and imetit (**2**). Immepip (**3**) fulfils the criteria for a selective histamine H_3 agonist, displaying low affinity for the H_1- and the H_2 receptor. It can be concluded that immepip (**3**) can be useful as a pharmacological tool and perhaps as a therapeutical agent, but also, because of its distinctive structure, for SAR- and Molecular Modelling studies.

Introduction

It has been shown that the presynaptic histamine H_3 receptor[5] regulates not only the release and synthesis of histamine, but also the release of other neurotransmitters[26,41] and can be regarded as a potential target for new therapeutics.[17,54] Until now only a few potent and selective agonists for the histamine H_3 receptor have been described. Methylation of the side chain of histamine has resulted in agonists like N^{α}-methylhistamine[5] and the chiral agonists (R)-α-methylhistamine[6] (**1**) and (R)α,(S)β-dimethylhistamine.[28,29] Out of this series of methylated histamine analogues, the (R)-enantiomer of α-methylhistamine **1** has been used extensively as a pharmacological tool. Recently the non-chiral histamine H_3 agonist, imetit (**2**) has been described.[37-40] This agonist is different from histamine and its methylated analogues, because it has a planar basic isothiourea group instead of an amino group. Imetit (**2**) and (R)-α-methylhistamine (**1**) are equipotent on the H_3 receptor as reported by Van der Goot *et al.* and Howson *et al.* on the inhibition of the electrically evoked twitches of the guinea pig ileum (jejunum).[37,38]

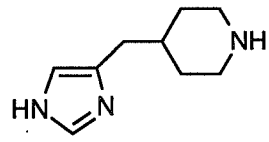


1, (R)-α-methylhistamine



2, imetit

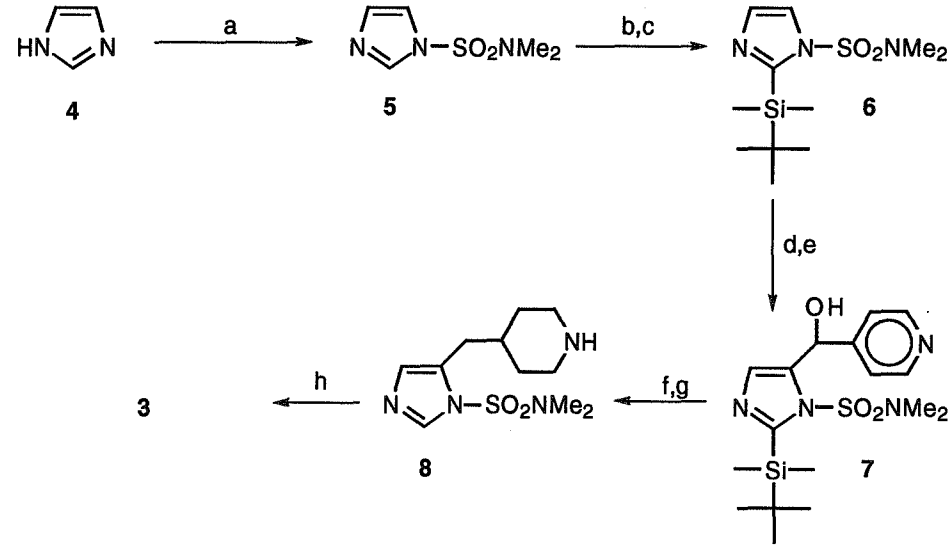
We now describe a new, potent and selective non-chiral histamine H_3 agonist, 4-(1*H*-imidazol-4-ylmethyl)piperidine (immepip) (**3**) as prepared from a series of histamine analogues[47] in which we incorporated the amino group in various ring structures, in order to obtain more information about the influence and the optimal location of the amino group relative to the imidazole ring. For immepip (**3**) the alkyl side chain was extended to a length of 4 methylene groups and the amino group was incorporated in a piperidine ring.



3, immepip

Chemistry

Immepip (**3**) was synthesized by the direct coupling of 4-pyridinecarboxaldehyde to the 5-position of a suitable 1,2-diprotected imidazole **6** by lithiation (Scheme I) (see also Chapter 5 and 9).[121] The hydroxyl group of **7** was removed by acylation and subsequent hydrogenation at 50 atm using Pd/C as a catalyst. Under these conditions the pyridine ring is also reduced to a piperidine ring and the *tert*-butyldimethylsilyl protecting group is hydrolyzed. After removal of the *N,N*-dimethylsulfonamide protecting group, immepip (**3**) was isolated as the dihydrobromide (overall yield was 70%). The reaction sequence has also been performed, using the trimethylsilyl group (cheaper) for protection of the 2-position of the imidazole ring (overall yield was 40%).



Scheme I Reagents used : (a) *N,N*-dimethylsulfamoylchloride, Et_3N, toluene. (b) *n*-BuLi, THF, -70°C. (c) *tert*-butyldimethylsilyl chloride. (d) *n*-BuLi, THF, -70°C. (e) 4-pyridinecarboxaldehyde. (f) DBU, Ac_2O. (g) H_2, Pd/C, 50 atm. (h) 30% HBr, reflux.

Pharmacology

The H_3 activity was functionally determined, on an *in vitro* test system based on the concentration dependent inhibition of electrically evoked twitches of isolated guinea pig jejunum segments by histamine H_3 agonists (see Chapter 3).[15]
The binding affinity for the H_1 receptor was determined by the displacement of [^{3}H]mepyramine, bound to membranes of CHO cells expressing guinea pig H_1 receptors.[122]
The binding affinity for the H_2 receptor was established by the displacement of [^{125}I]iodoaminopotentidine, bound to membranes of CHO cells expressing human H_2 receptors.[123]

Results and Discussion

Average concentration response curves (CRC's) for immepip (**3**) and (R)-α-Methylhistamine (**1**) (for comparison) are shown in Figure 1.

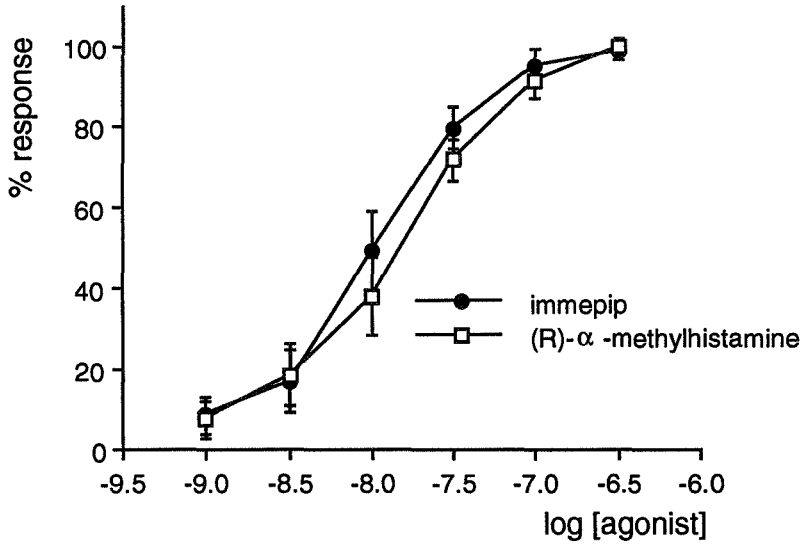


Figure 1 Concentration response curves for immepip (n = 8) and (R)-α-methylhistamine (n = 22), constructed from the inhibition of the electrically evoked twitches of the guinea pig jejunum.[15] n Represents the number of animals used. Values shown in the graph are expressed as mean ± SD

From this graph it is clear that immepip (**3**) is equipotent as (or even slightly more active than) (R)-α-methylhistamine (**1**) on the H_3 receptor. The pD_2 value for immepip (**3**) as determined on jejunum preparations of eight different animals was 8.0 ± 0.1 (mean ± SD). For comparison, (R)-α-methylhistamine (**1**) has a pD_2 value of 7.8 ± 0.2 (n = 22) on this test system. The H_3 antagonist thioperamide caused a rightward parallel shift of the CRC for immepip (**3**). The pA_2 value of thioperamide, using immepip (**3**) as an agonist, was 8.2 ± 0.2 with a Schild slope of 0.8 ± 0.1 (n = 3) (not significantly different from unity). This is slightly lower than the pA_2 value of thioperamide, obtained using (R)-α-methylhistamine (**1**) on this assay.[15] This lower affinity has also been reported, using imetit (**2**) as agonist.[39]

The potent agonistic activity of immepip (**3**) on the H_3 receptor, was confirmed in radioligand binding studies (Figure 2).

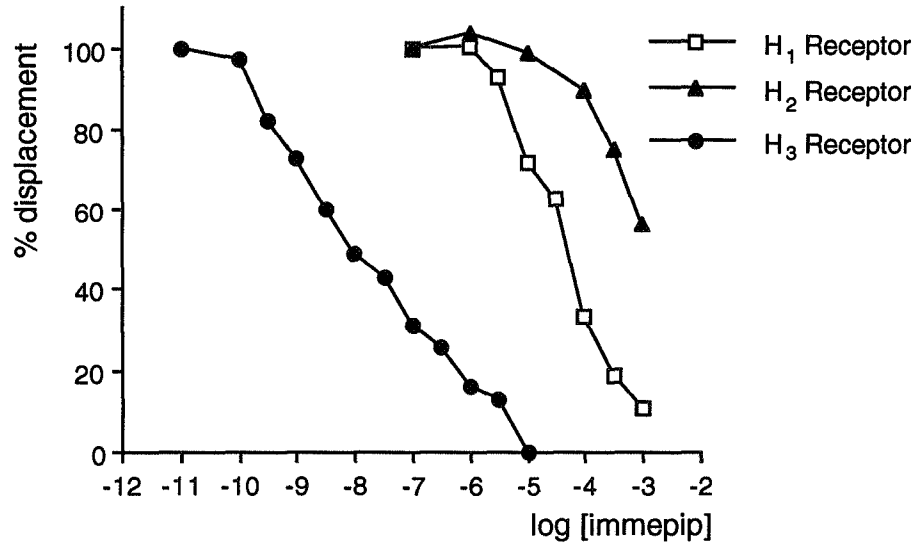


Figure 2 Receptor selectivity of immepip as measured by radioligand binding studies. Displacement of the specific binding ligands in representative experiments is shown. Competition of immepip with [^{3}H]mepyramine binding to membranes of CHO cells expressing guinea pig H_1 receptors,[122] [^{125}I]iodoaminopotentidine binding to membranes of CHO cells expressing human H_2 receptors[123,124] and [^{125}I]iodophenpropit binding to membranes from rat cortex[50] was measured in at least three independent experiments, performed in triplicate.

Displacement of the H_3 antagonist [^{125}I]iodophenpropit[49,50] binding to rat cortex membranes resulted in shallow displacement curves for immepip (**3**). Computer analysis of these data reveals two binding sites (using the program LIGAND).[125] This is in agreement with the described displacement curves for other H_3 agonists and indicative for the interaction of the H_3 receptor with a G-protein. The K_H and the K_L for immepip (**3**) on the H_3 receptor, are 2.7 ± 0.5 nM and 1.01 ± 0.2 μM respectively. For comparison (R)-α-methylhistamine (**1**) showed a K_H and a K_L of 4.3 ± 3.4 nM and 0.22 ± 0.15 μM respectively, on the same assay.[50] From figure 2 it is also clear that immepip (**3**) is highly selective for the H_3 receptor. The pK_i of immepip (**3**) for the guinea pig H_1 receptor was 4.79 ± 0.10 (using [^{3}H]mepyramine as a radioligand),[122] whereas its affinity for the human H_2 receptor[124] was too low to be determined accurately (using [^{125}I]iodoaminopotentidine as a radioligand; $pK_i < 3.5$).

If we compare the structure of the methylated histamine analogues, imetit (**2**) and immepip (**3**), some interesting observations can be made. The amino group of histamine and its methylated analogues is protonated at physiological pH[126] and is located at a distance of 2 methylene groups (≈ 4.5 Å) away from the imidazole ring. This ammonium group could interact with a carboxylate group in the receptor, as postulated for the H_2 receptor.[127,128] The isothiourea group of imetit (**2**), is also protonated at a pH of 7.4.[40] This means that the isothiouronium group can also interact with a carboxylate group. However, since only the imino nitrogen of the isothiourea group can be protonated, the distance between the imidazole ring and the hydrogen donating nitrogens is not 2 methylene groups (≈ 4.5 Å) as in histamine and its methylated analogues, but longer (≈ 8 Å). For immepip (**3**) it is obvious that the proton donating ammonium group is located at a distance of 4 methylene groups from the imidazole ring (≈ 7.5 Å). These observations make immepip (**3**), together with imetit (**2**) and the methylated analogues a valuable tool in Molecular Modelling studies.

It can be concluded that immepip (**3**) is a new and selective histamine H_3 agonist, equipotent as (R)-α-methylhistamine (**1**) and imetit (**2**), which can be useful as a pharmacological tool and perhaps as a therapeutical agent, but also, because of its distinctive structure, for SAR- and Molecular Modelling studies.

Experimental Section

Chemistry

*4-[1-(*N,N-*Dimethylsulfamoyl)-2-*tert-*butyldimethylsilyl-imidazol-5-ylhydroxymethyl]pyridine* (**7**)

10.0 g (57 mmol) 1-(*N,N*-Dimethylsulfamoyl)imidazole (**5**) was dissolved in dry THF (200 ml) under an atmosphere of dry nitrogen and cooled to -70°C. *n*-Butyllithium in hexane (36.0 ml, 58 mmol) was added dropwise (temperature should not exceed -65°C). After 15 min, a solution of *tert*-butyl-dimethylsilyl chloride (9.0 g, 60 mmol) in dry THF (20 ml) was added and the solution was stirred at room temperature for 1 h. The mixture was cooled to -70°C again and *n*-butyllithium in hexane (36.0 ml, 58 mmol) was added dropwise (temperature should not exceed -65°C). After 0.5 h, a solution of 4-pyridinecarboxaldehyde (6.5 g, 61 mmol) in dry THF (15 ml) was added gradually and the cooling bath was removed. After the reacting mixture reached room temperature, the solution was poured into water (200 ml) and the THF was removed under reduced pressure. The product was extracted with ether (3 x 150 ml), dried (Na_2SO_4) and concentrated in vacuo. 17.0 g (75%) Of a yellow solid remained.

^{1}H NMR ($CDCl_3$) : δ 0.40 (s, 6H, $SiCH_3$), 1.00 (s, 9H, CCH_3), 2.82 (s, 6H, NCH_3), 6.10 (s, 1H, CH), 6.68 (s, 1H, Im-4H), 7.37 (d, 2H, J = 7Hz, Pyr-3,5-H), 8.12 (d, 2H, J = 7Hz, Pyr-2,6-H) ppm.

*4-[1-(*N,N-*Dimethylsulfamoyl)imidazol-5-ylmethyl]piperidine* (**8**)

11.53 g (29 mmol) **7** Was dissolved in 150 ml of acetonitrile. 5.2 ml (35 mmol) DBU was added and 3.6 ml acetic anhydride (38 mmol) was added after 10 min. After 15 min. stirring at ambient temperature, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 and washed with H_2O (three times). The organic layer was dried on Na_2SO_4 and concentrated removed under reduced pressure. Yield 12.7 g (100%).

^{1}H NMR ($CDCl_3$) : δ 0.38 (s, 6H, $SiCH_3$), 0.98 (s, 9H, CCH_3), 2.12 (s, 3H, $OCCH_3$), 2.80 (s, 6H, NCH_3), 6.98 (s, 1H, CH), 7.10 (s, 1H, Im-4H), 7.24 (d, 2H, J = 7Hz, Pyr-3,5-H), 8.60 (d, 2H, J = 7Hz, Pyr-2,6-H) ppm.

7.3 g Of this product (16.7 mmol) was dissolved in 50 ml acetic acid. 0.7 g 10% Pd/C Was added and this mixture was hydrogenated for 16 hours under hydrogen pressure (50 atm) in an autoclave. The reaction mixture was filtrated over a short celite column, concentrated *in vacuo* and washed with absolute ethanol (2 times with 20 ml.). The hydrogenation was incomplete and repeated once more as described above. This time the hydrogenation was complete. Yield 5.5 g (99%) (acetate salt)
The residue was dissolved in 100 ml H_2O and the pH was raised to 12 by the addition of K_2CO_3. The basic water layer was extracted with CH_2Cl_2 (3 x 100 ml). The organic layers were combined, dried on Na_2SO_4 and concentrated *in vacuo*.

^{1}H NMR ($CDCl_3$) : δ 0.95-1.28 (m, 2H, Pip-3,5-H_{ax}), 1.59-1.81 (m, 3H, Pip-3,5-H_{eq} + Pip-4H), 2.50 (m, 2H, Pip-2,6-H_{ax}), 2.60 (d, 2H, J = 7Hz, CH_2), 2.83 (s, 6H, NCH_3), 3.02 (m, 2H, Pip-2,6-H_{eq}), 6.78 (s, 1H, Im-4H), 7.82 (s, 1H, Im-2H) ppm.

^{13}C NMR ($CDCl_3$) : δ 32.0, 33.1, 35.5, 37.8, 46.3, 129.2, 130.3, 138.0 ppm.

4-(1H-Imidazol-4-ylmethyl)piperidine dihydrobromide (immepip) (**3**)

5.5 g (16.6 mmol) **8** Was refluxed in 30% HBr for 16 hours. The solution was concentrated under reduced pressure and the residue was dissolved and refluxed in absolute ethanol for one hour. This mixture was concentrated in vacuo and washed with acetone (three times). 5.1 g (94%) White crystals were collected. Melting point 221.1-222.7 °C.

^{1}H NMR (D_2O) : δ 1.48 (qd, 2H, J = 13, 3 Hz, Pip-3,5-H_{ax}), 1.94 (dm, 2H, J = 13 Hz, Pip-3,5-H_{eq}), 2.00 (m, 1H, Pip-4-H), 2.72 (d, 2H, J = 7 Hz, CH_2), 2.98 (tm, 2H, J = 13 Hz, Pip-2,6-H_{ax}), 3.41 (dm, 2H, J = 13 Hz, Pip-2,6-H_{eq}), 7.28 (s, 1H, Im-4(5)H), 8.59 (s, 1H, Im-2H) ppm.

^{13}C NMR (D_2O) : δ 29.2, 31.3, 34.5, 45.3, 117.8, 132.4, 134.5 ppm.

High resolution mass : m/z 165.1265; calcd. for $C_9H_{15}N_3$: 165.1266

Anal. ($C_9H_{15}N_3$•2HBr) : 32.86 %C, 5.23 %H, 12.6 %N, 49.0 %Br;
calcd : 33.05 %C, 5.24 %H, 12.8 %N, 49.0 %Br.

Pharmacology

The histamine H_3 activity of immepip (**3**), was determined on an *in vitro* assay, based on the inhibitory effect of histamine H_3 agonists on electrically evoked twitches (induced by endogenous acetylcholine release) of guinea pig jejunum preparations (see Chapter 3).[15] The addition of cumulative concentrations of immepip (**3**), results in a concentration-dependent inhibition of these evoked twitches, from which a concentration-response curve can be constructed. The binding affinity of the described compounds for the H_1 receptor was determined in at least three independent experiments, performed in triplicate, by the displacement of [^{3}H]mepyramine, bound to membranes of CHO cells expressing guinea pig H_1 receptors.[122] The binding affinity of the described compounds for the H_2 receptor was established in at least three independent experiments, performed in triplicate, by the displacement of [^{125}I]iodoaminopotentidine, bound to membranes of CHO cells expressing human H_2 receptors.[123]

Acknowledgements We wish to thank Mr. T.S. Wu for the determination of the binding affinity of immepip on the H_3 receptor. The research of Dr. R. Leurs has been made possible by a fellowship of the Royal Netherlands Academy of Arts and Sciences.

Chapter 7

Homologues of Histamine as Histamine H_3 Receptor Antagonists: A New Potent and Selective H_3 Antagonist 4(5)-(5-Aminopentyl)-1*H*-imidazole (Impentamine)

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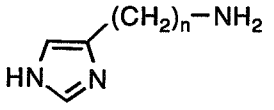
Abstract

The influence of alkyl chain length variation on the histamine H_3 receptor activity of histamine homologues **1** was investigated. A series of 4(5)-(ω-aminoalkyl)-1*H*-imidazoles **1** was prepared with an alkyl chain length varying from one methylene group to 10 methylene groups. Besides the H_3 activity, the affinities of these compounds for the H_1 and H_2 receptors were determined. The ethylene chain of histamine is optimal for agonistic activity on all three histamine receptor subtypes. For the H_3 receptor, elongation of the alkyl chain from three methylene groups on leads to compounds with antagonistic properties. 4(5)-(5-Aminopentyl)-1*H*-imidazole (impentamine, **1e**) is the most potent and selective H_3 antagonist from this series of 4(5)-(ω-aminoalkyl)-1*H*-imidazoles **1**, with a pA_2 value of 8.4 (on guinea pig jejunum). A specific antagonistic binding site for this compound is proposed.

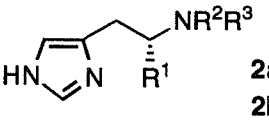
Introduction

The histamine H_3 receptor has been described to play a role as a general regulatory receptor system, regulating not only the release and synthesis of histamine, but also the release of other neurotransmitters.[71] Therefore, this receptor can be regarded as a potential target for new therapeutics.[17,26,41] To characterize its role in physiology however, there is still a need for selective, preferably nontoxic ligands. The most potent H_3 ligands described so far are the H_3 agonist imetit (**3a**) and the H_3 antagonist clobenpropit (**3c**), both containing an isothiourea group. We directed part of our research to the development of non-isothiourea-containing selective histamine H_3 receptor ligands using the natural agonist histamine (**1b**) as a 'lead'.

A reasonable number of analogues of histamine and their effect on the histamine H_3 receptor, have been described in literature. Most successful alterations of the structure of histamine have been performed by functionalization of the ethylene chain, resulting in, for instance, the potent and selective H_3 agonist (R)-α-methylhistamine (**2a**).[6] Alkylations of the amino group of histamine are tolerated when small alkyl groups are used. N^{α}-Methylhistamine (**2b**) and N^{α},N^{α}-dimethylhistamine (**2c**) are slightly more active than histamine (**1b**).[5] Replacement of the amino group with other polar cationic groups resulted in the potent and selective H_3 agonist imetit (**3a**).[37-40] Replacement of the imidazole ring of histamine and its analogues by other heterocycles or structural imidazole mimics resulted in less active (e.g., betahistine acts as a weak H_3 antagonist), but more frequently in inactive compounds.[26,41] Substituents on the imidazole ring of histamine (**1b**) are also not allowed (e.g., *N*-methyl-, 2-methyl- and 4(5)-methylhistamine are inactive).[5]

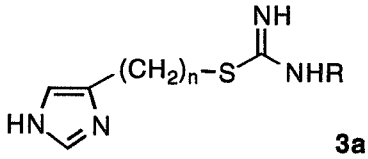


1 n =1-10



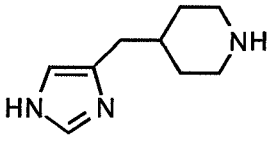
2

2a R^1=Me; R^2=R^3=H
2b R^1=R^2=H; R^3=Me
2c R^1=H; R^2=R^3=Me



3

3a n=2; R=H; imetit
3b n=3; R=H; VUF 8328
3c n=3; R=4-Cl-benzyl; clobenpropit



4; immepip

Figure 1. Discussed structures.

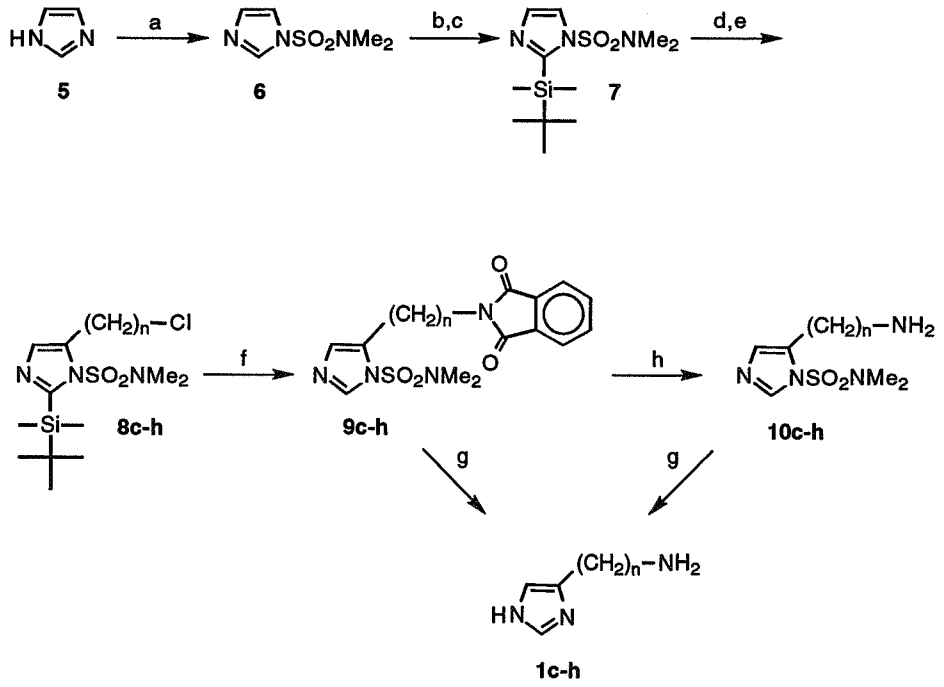
Recently, we reported a new potent and selective agonist for the H_3 receptor, which is not an ethylene chain analogue of histamine (**1b**) (see Chapter 6).[42] This compound, immepip (**4**), has an aminobutylene chain, incorporated in a piperidine ring; yet in activity (pD_2 = 8.0 on guinea pig jejunum), it is comparable to (R)-α-methylhistamine (**2a**) (pD_2 value of 7.8 on the same test system).[15] If we compare the structures of histamine (**1b**) and immepip (**4**), it is clear that the amino groups in both structures are separated by alkyl spacers different in length. The amino group of histamine (**1b**) is located at a distance of two methylene groups (≈4.5 Å) from the imidazole ring, compared to a distance of four methylene groups (≈7.5 Å) in immepip (**4**). Since immepip (**4**) is more potent than histamine (**1b**) on this receptor, we wanted to investigate the H_3 effect of chain length variation of the alkyl spacer in a series of histamine homologues.

In literature, not much is known about the histamine H_3 activity of 4(5)-(ω-aminoalkyl)-1*H*-imidazoles **1**. It has been reported that a histamine homologue with a chain length of only one methylene group (**1a**) is inactive on the H_3 receptor (guinea pig ileum).[41] Elongation of the alkyl chain to three methylene groups (**1c**) also has been described by Lipp[26] to result in a compound with no H_3 agonistic properties (rat cortex) and by Leurs[41] to result in a compound with weak H_3 antagonistic properties (pA_2 value of 6.0 on rat cortex).

In order to investigate the effect of the chain length of the alkyl spacer of histamine homologues **1** on the histamine H_3 receptor activity, we have prepared a series of 4(5)-(ω-aminoalkyl)-1*H*-imidazoles **1** with an alkyl chain length varying from one methylene group to 10 methylene groups. We determined the H_3 activity of these compounds functionally on an *in vitro* test system using guinea pig jejunum preparations. In order to establish the selectivity of these compounds for the H_3 receptor, we determined the affinities of the 4(5)-(ω-aminoalkyl)-1*H*-imidazoles **1** on the H_1- and the H_2 receptor as well.

Chemistry

The 4(5)-(ω-aminoalkyl)-1*H*-imidazoles **1c-h** were prepared by a method described earlier by our group (Scheme 1) (see also Chapter 5).[121] This method is based on the direct coupling of an alkyl chain to the imidazole ring, by lithiation of a suitable 1,2-diprotected imidazole **7** and subsequent treatment with 1-chloro-ω-iodoalkanes. The ω-chloro group of the resulting products **8c-h** can be converted into an amino group via the Gabriel synthesis.[119] The protecting group on the 2-position of the imidazole ring (the *tert*-butyldimethylsilyl group) hydrolyses under the basic conditions of the Gabriel synthesis. The free amino group can be obtained, either by hydrolysis of the phthalimide group under acidic conditions resulting directly in compounds **1c-h** or by reaction with hydrazine resulting in compounds **10c-h**. The advantage of hydrolysis of the phthalimide group under acidic conditions is that the N-protecting group on the imidazole ring can be removed in the same step. If however the protecting group is required in further reactions (like addition reactions on the amino group), hydrazine should be used, because the protecting group is unaffected under these conditions.



Scheme I. Reagents used: (a) *N,N*-Dimethylsulfamoyl chloride, Et_3N, toluene; (b) *n*-BuLi, THF, -70°C; (c) *tert*-butyldimethylsilyl chloride; (d) *n*-BuLi, THF, -70°C; (e) 1-chloro-ω-iodoalkane; (f) potassium phthalimide, DMF, +70°C; (g) 30% HBr, reflux; (h) $H_2NNH_2 \cdot H_2O$, EtOH.

After conversion of the chloro group into an amino group and removal of the protecting groups, the desired histamine analogues **1c-h** were obtained. Using this method we have prepared histamine analogues with an alkyl chain of up to 10 methylene groups. Some of the compounds were isolated as salts of oxalic acid because of better stability and isolation. We observed no problems using oxalates in our *in vitro* test system.

Pharmacology

The H_3 activity of the compounds was determined on an *in vitro* test system, on the basis of the concentration-dependent inhibitory effect of histamine H_3 agonists on the electrically evoked contractile response of isolated guinea pig jejunum segments (see Chapter 3).[15]

The binding affinity for the H_1 receptor was determined by the displacement of [^{3}H]mepyramine, bound to membranes of CHO cells expressing guinea pig H_1 receptors.[122]

The binding affinity for the H_2 receptor was established by the displacement of [^{125}I]iodoaminopotentidine, bound to membranes of CHO cells expressing human H_2 receptors.[123]

Results and Discussion

All the compounds have been tested for H_3 agonism and H_3 antagonism on guinea pig jejunum. From the results shown in Table 1 (see also Figure 2), it is clear that variation of the alkyl chain length in 4(5)-(ω-aminoalkyl)-1*H*-imidazoles **1** has a drastic effect on the activity of these compounds on the histamine H_3 receptor. The histamine homologue with an alkyl chain shortened to one methylene group, VUF 8319 (**1a**), is inactive on this receptor. The ethylene chain of histamine (**1b**) is clearly optimal for activation of the H_3 receptor in the series of 4(5)-(ω-aminoalkyl)-1*H*-imidazoles **1**, since it is the only compound in this series with H_3 agonistic activity. Elongation of the alkyl chain results in compounds with a surprisingly high antagonistic activity on the histamine H_3 receptor (no agonistic activity was observed on the used test system). 4(5)-(3-Aminopropyl)-1*H*-imidazole (VUF 8326, **1c**), for example, possesses moderate antagonistic activity, but the pentyl homologue of histamine, 4(5)-(5-aminopentyl)-1*H*-imidazole (impentamine or VUF 4702, **1e**), is a potent H_3 antagonist with a pA_2 value of 8.4 on the described *in vitro* test system. A further increase in the chain length to eight methylene groups, results in a decrease of antagonistic activity to a pA_2 value of 6.0, which does not change on further lengthening (up to 10 methylene groups).

Table 1. H_3 antagonistic activity of the Histamine homologues **1** as tested on the *in vitro* test system on the guinea pig jejunum.

Compound	Name or Code [a]	n [b]	pA_2 H_3 [c]	Slope [d]	N [e]
1a	VUF 8319	1	< 3		4
1b	histamine	2	$pD_2 = 6.2 \pm 0.3$	–	4
1c	VUF 8326	3	5.9 ± 0.3	0.9 ± 0.3	4
1d	VUF 4701	4	7.7 ± 0.2	0.8 ± 0.1	4
1e	VUF 4702	5	8.4 ± 0.2	1.1 ± 0.1	7
1f	VUF 4732	6	7.8 ± 0.2	1.2 ± 0.1	7
1g	VUF 4733	8	6.0 ± 0.2	1.2 ± 0.1	6
1h	VUF 4734	10	6.0 ± 0.3	0.9 ± 0.2	7

[a] Compound code number.
[b] Alkyl chain length of **1** (number of methylene units).
[c] Antagonistic parameter as determined on the described *in vitro* H_3 assay representing the negative logarithm of the abscissal intercept from the Schild plot ± SD.
[d] Slope of Schild plot ± SD, not significantly different from unity.
[e] Number of different animal preparations.

From these results it is clear that in a series of 4(5)-(ω-aminoalkyl)-1*H*-imidazoles **1** the ethylene chain is essential for activation of the histamine H_3 receptor. The optimal chain length (through bonds) for antagonistic activity in this series seems to be five methylene groups (between the primary amino group and the imidazole ring). These results indicate that both the imidazole ring and the amino group play an important role in the activation mechanism of the H_3 receptor. It seems likely that there are specific binding sites for the imidazole ring and the protonated amine (at physiological pH) in the receptor. As soon as histamine binds to these sites, the receptor is activated. Apparently the higher alkyl analogues can not fulfil these requirements for activation (probably for steric reasons).

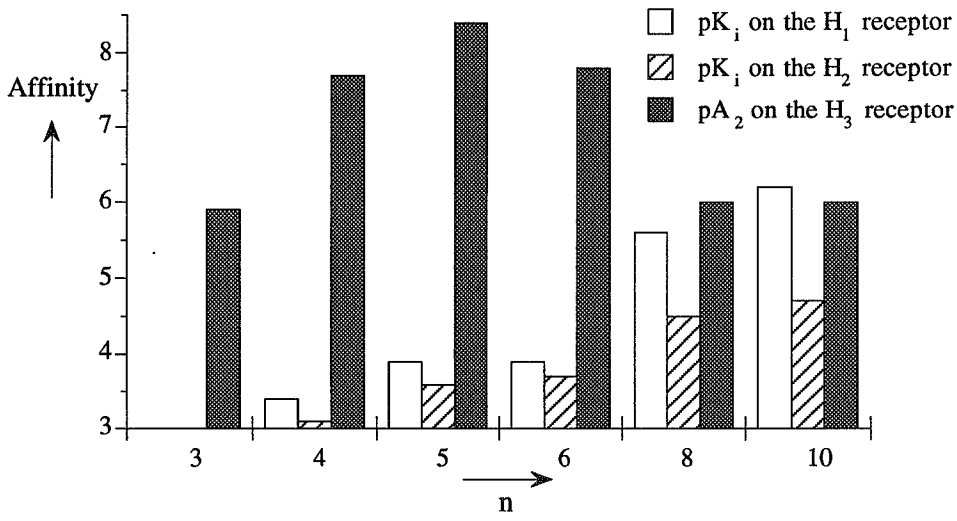


Figure 2. The influence of the alkyl chain length (n) of the histamine homologues **1** on the affinity on the H_1- and the H_2 receptor and the antagonistic activity on the H_3 receptor.

The observation that the histamine homologue with an alkyl chain comprising five methylene groups (impentamine, **1e**) has a much higher affinity than for instance the propyl homologue (VUF 8326, **1c**) or the octyl homologue (VUF 4733, **1g**) seems to indicate an additional binding site, for specific antagonists, in the H_3 receptor. Occupation of this additional binding site by either the ammonium group (at physiological pH) or the imidazole ring of impentamine (**1e**) increases the affinity, but the receptor is not activated; in figure 3 this speculation is represented in a graphical manner. In this respect it is interesting to note that similar observations can be made in the series of analogues of imetit (**3a**), described by Van der Goot.[37] Imetit (**3a**), is a potent agonist for the H_3 receptor (pD_2 value of 8.1 on guinea pig ileum), but when the ethylene chain between the imidazole ring and the isothiourea group is elongated to a propylene chain, a potent H_3 antagonist, VUF 8328 (**3b**), is obtained (pA_2 value of 8.0 on guinea pig ileum). The distance (through bonds) between the imidazole ring and the protonated nitrogen is comparable for both the elongated imetit analogue VUF 8328 (**3b**) and impentamine (**1e**). This observation supports the idea of an extra binding site for antagonists to which either a protonated cationic nitro-

gen or the imidazole ring can bind, thereby enhancing the affinity, but not activating the H_3 receptor.

Figure 3. Simplistic graphical representation of a possible additional binding site for antagonists in the H_3 receptor, which can be occupied by either the protonated cationic nitrogen (triangle) or the imidazole ring (circle) of impentamine (**1e**) and VUF 8328 (**3b**). **I** : An H_3 agonist activates the receptor by binding to the 'active site'. **II** : An H_3 antagonist (like impentamine (**1e**) or VUF 8328 (**3b**)) binds with the protonated cationic nitrogen (**IIa**) or the imidazole ring (**IIb**) to an additional specific antagonistic binding site. No activation occurs.

Since introduction of substituents (aralkyl groups) on the isothiourea group of VUF 8328 (**3b**) resulted in even more potent antagonists, like clobenpropit (**3c**) (pA_2 value of 9.9 on guinea pig ileum), we are currently investigating the effect of introduction of these types of substituents on the amino group of the described 4(5)-(ω-aminoalkyl)-1*H*-imidazoles **1**, on their H_3 activity.

A rather striking observation can be made when comparing the butyl homologue of histamine VUF 4701 (**1d**) and immepip (**4**), which can be considered as its rigid analogue. VUF 4701 (**1d**) is a rather potent antagonist, whereas immepip (**4**) is a potent and selective agonist on the H_3 receptor. The difference in activity may be due to specific steric and/or conformational effects.

Chain length variation in a series of 4(5)-(ω-aminoalkyl)-1*H*-imidazoles **1** has a large effect on the affinity of these compounds for the H_1- and the H_2 receptor as well. It has been reported already more than 60 years ago[115] that there is a marked decrease in the effect of the propylene- (**1c**) and butylene (**1d**) homologues of histamine on the H_1 activity. For the H_2 receptor, both shortening to the methylene chain (**1a**) and elongation to the propylene chain (**1c**) have been reported to result in histamine

analogues with an agonistic activity less than 0.5% of the activity of histamine (**1b**).[129] This is in agreement with the affinities for the H_1 and H_2 receptors of the 4(5)-(ω-aminoalkyl)-1*H*-imidazoles **1** that we have determined.

Table 2. Binding affinity of the Histamine analogues **1** on the H_1- and the H_2 receptor.

Compound	Name or Code [a]	n [b]	pK_i H_1 [c]	pK_i H_2 [d]
1a	VUF 8319	1	< 3	< 3
1b	histamine	2	4.9	4.4
1c	VUF 8326	3	< 3	< 3
1d	VUF 4701	4	3.4 ± 0.1	3.1 ± 0.1
1e	VUF 4702	5	3.9 ± 0.1	3.6 ± 0.1
1f	VUF 4732	6	3.9 ± 0.1	3.7 ± 0.1
1g	VUF 4733	8	5.6 ± 0.1	4.5 ± 0.1
1h	VUF 4734	10	6.2 ± 0.1	4.7 ± 0.1

[a] Compound code number.
[b] Alkyl chain length of **1** (number of methylene units).
[c] Negative logarithm value of the binding affinity for the histamine H_1 receptor ± SEM.
[d] Negative logarithm value of the binding affinity for the histamine H_2 receptor ± SEM.

From Table 2 and Figure 2 it can be seen that variation in the length of the alkyl spacer of histamine results in compounds with a low affinity for the H_1- and the H_2 receptor, although the affinity increases with increasing chain length. The histamine homologue with a decyl chain (**1h**) has a reasonable affinity for the H_1 receptor (pK_i = 6.2), which was confirmed to be a weak antagonist by a conventional *in vitro* assay on the guinea pig ileum (not shown).

We conclude that the ethylene chain is the optimal spacer in 4(5)-(ω-aminoalkyl)-1*H*-imidazoles **1** for agonistic activity on all three known histamine receptor subtypes. For the histamine H_1 and H_2 receptors, variation of the alkyl chain length only results in compounds with a low affinity, although the affinity increases with increasing chain length. The histamine analogue with a decyl chain (**1h**) is even a moderate H_1 antagonist, which is rather surprising for an imidazole-based structure.

For the H_3 receptor, the effect of chain elongation of histamine is most pronounced, resulting e.g., in the potent and selective H_3 antagonist 4(5)-(5-aminopentyl)-1*H*-imidazole (impentamine, **1e**). This high activity suggests an additional specific antagonistic binding site in the H_3 receptor to which either the ammonium group or the imidazole ring can bind, leading to enhanced affinity but not to activation.

Experimental Section

Chemistry

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC-200 (200 MHz) spectrometer with tetramethylsilane or sodium 3-(trimethylsilyl)propionate as an internal standard. Mass spectra were recorded on a Finnigan MAT-90. Melting points were measured on a Mettler FP-5 + FP-52 instrument and are uncorrected. THF was distilled from $LiAlH_4$, DMF was dried by passage through a column packed with Al_2O_3, and acetone was distilled from K_2CO_3. *n*-Butyllithium was purchased from Janssen Chimica as a 15% solution in hexane.

The 1-chloro-ω-iodoalkanes were prepared by refluxing the corresponding 1,ω-dichloroalkanes with 1 equiv of sodium iodide in acetone and purification of the product by distillation.[120]

1-(*N,N*-Dimethylsulfamoyl)imidazole **6** was synthesized by the method described by Chadwick and Ngochindo,[90] with the exception that toluene was used as a solvent instead of benzene.

4(5)-(1-Aminomethyl)-1*H*-imidazole (**1a**) was prepared according to literature procedure.[130] Histamine dihydrochloride (**1b**) was purchased from Janssen Chimica. 4(5)-(3-Aminopropyl)-1*H*-imidazole dihydrobromide (**1c**), 4(5)-(4-aminobutyl)-1*H*-imidazole dihydrobromide (**1d**), and 4(5)-(5-aminopentyl)-1*H*-imidazole dihydrobromide (**1e**) were prepared as described earlier by our group (see Chapter 3).[121]

*2-(*tert*-Butyldimethylsilyl)-5-(6-chlorohexyl)-1-(*N,N*-dimethylsulfamoyl)imidazole (8f)*

1-(*N,N*-Dimethylsulfamoyl)imidazole (**6**) (26.0 g, 0.15 mol) was dissolved in dry THF (500 ml) under an atmosphere of dry nitrogen and cooled to -70°C. *n*-Butyllithium in hexane (100 ml, 0.16 mol) was added dropwise (internal temperature did not exceed -65°C). After 15 min, a solution of *tert*-butyldimethylsilyl chloride (25.0 g, 0.17 mol) in dry THF (50 ml) was added (internal temperature did not exceed -65°C) and the solution was allowed to warm to room temperature and stirred for an additional hour. The mixture was cooled to -70°C again, and *n*-butyllithium in hexane (100 ml, 0.16 mol) was added dropwise (internal temperature did not exceed -65°C). After 0.5 h, a solution of 1-chloro-6-iodohexane (44.3 g, 0.17 mol) in dry THF (50 ml) was added gradually and the mixture was allowed to (slowly) warm to room temperature overnight. The reaction mixture was poured into water (200 ml), and the THF was removed under reduced pressure. The product was extracted with

$CHCl_3$ (3 x 150 ml), dried (Na_2SO_4) and concentrated in vacuo. 73.2 g Of a viscous orange coloured oil was obtained which was used without further purification. ^1H NMR Analysis of the oil showed complete coupling of the 6-chlorohexyl chain to the 1,2-diprotected imidazole **7**.

^1H NMR ($CDCl_3$): δ 0.39 (s, 6H, $Si(C\underline{H}_3)_2$), 1.01 (s, 9H, $C(C\underline{H}_3)_3$), 1.38 (m, 4H, central $C\underline{H}_2$'s), 1.80 (m, 4H, 2*$C\underline{H}_2$), 2.71 (t, 2H, J = 8 Hz, imidazole-5-$C\underline{H}_2$), 2.88 (s, 6H, $N(C\underline{H}_3)_2$), 3.55 (t, 2H, J = 7 Hz, $C\underline{H}_2Cl$), 6.93 (s, 1H, imidazole-4H) ppm.

*2-(*tert*-Butyldimethylsilyl)-5-(8-chlorooctyl)-1-(*N,N*-dimethylsulfamoyl)imidazole (8g)*

The same procedure as for the preparation of **8f** was employed, using 9.64 g (55 mmol) 1-(*N,N*-dimethylsulfamoyl)imidazole (**6**) in 100 ml dry THF, 37.5 ml (60 mmol) *n*-butyllithium solution, 9.0 g (60 mmol) *tert*-butyldimethylsilyl chloride and 18.0 g (65 mmol) 1-chloro-8-iodooctane. 32.6 g Of a viscous orange oil was obtained which was used without further purification. ^1H NMR Analysis of the oil showed complete coupling of the 8-chlorooctyl chain to the 1,2-diprotected imidazole **7**.

^1H NMR ($CDCl_3$): δ 0.39 (s, 6H, $Si(C\underline{H}_3)_2$), 1.01 (s, 9H, $C(C\underline{H}_3)_3$), 1.37 (m, 8H, central $C\underline{H}_2$'s), 1.37 (m, 4H, 2*$C\underline{H}_2$), 2.71 (t, 2H, J = 8 Hz, imidazole-5-$C\underline{H}_2$), 2.85 (s, 6H, $N(C\underline{H}_3)_2$), 3.55 (t, 2H, J = 7 Hz, $C\underline{H}_2Cl$), 6.95 (s, 1H, imidazole-4H) ppm.

*2-(*tert*-Butyldimethylsilyl)-5-(10-chlorodecyl)-1-(*N,N*-dimethylsulfamoyl)imidazole (8h)*

The same procedure as for the preparation of **8f** was employed, using 4.9 g (28 mmol) 1-(*N,N*-dimethylsulfamoyl)imidazole (**6**) in 100 ml dry THF, 20.0 ml (32 mmol) *n*-butyllithium solution, 4.7 g (31 mmol) *tert*-butyldimethylsilyl chloride and 9.2 g (31 mmol) 1-chloro-10-iododecane. 18.3 g Of a viscous orange oil was obtained which was used without further purification. ^1H NMR Analysis of the oil showed complete coupling of the 10-chlorodecyl chain to the 1,2-diprotected imidazole **7**.

^1H NMR ($CDCl_3$): δ 0.38 (s, 6H, $Si(C\underline{H}_3)_2$), 1.00 (s, 9H, $C(C\underline{H}_3)_3$), 1.32 (m, 12H, central $C\underline{H}_2$'s), 1.80 (m, 4H, 2*$C\underline{H}_2$), 2.73 (t, 2H, J = 8 Hz, imidazole-5-$C\underline{H}_2$), 2.85 (s, 6H, $N(C\underline{H}_3)_2$), 3.53 (t, 2H, J = 7 Hz, $C\underline{H}_2Cl$), 6.94 (s, 1H, imidazole-4H) ppm.

1-(N,N-Dimethylsulfamoyl)-5-(6-phthalimidohexyl)imidazole (9f)

The crude product **8f** (73.2 g) was dissolved together with potassium phthalimide (40.3 g, 0.2 mol) in DMF (900 ml) and the mixture was heated at 70°C. After 7 h the mixture was filtered and the solvent was evaporated under reduced pressure. The residue (86.8 g) was stirred in *iso*-propanol (400 ml) and the precipitate (beige powder) was removed by filtration (1,6-diphthalimidohexane). The filtrate was concentrated in vacuo and the residue was dissolved in $CHCl_3$ (150 ml), washed with H_2O (3 x 150 ml), dried (Na_2SO_4) and concentrated in vacuo. A dark brown oil remained (47.6 g), which was pure enough for further use. A portion of this oil was purified by flash chromatography with ethyl acetate as eluent (R_f = 0.4). The ^1H NMR spectrum of the purified oil indicated complete removal of the protective group on the 2-position.

^1H NMR ($CDCl_3$): δ 1.42 (m, 4H, central $C\underline{H}_2$'s), 1.69 (m, 4H, 2*$C\underline{H}_2$), 2.72 (t, 2H, J = 8 Hz, imidazole-5-$C\underline{H}_2$), 2.90 (s, 6H, $N(C\underline{H}_3)_2$), 3.70 (t, 2H, J = 7 Hz, $C\underline{H}_2$-phthalimide), 6.82 (s, 1H, imidazole-4H), 7.72 (m, 5H, phthalimide-H + imidazole-2H) ppm.

1-(N,N-Dimethylsulfamoyl)-5-(8-phthalimidooctyl)imidazole (9g)

The same procedure as for the preparation of **9f** was employed, using the crude product **8g** (32.6 g) in 150 ml DMF and 13.6 g (73 mmol) potassium phthalimide. After work up, a dark brown oil remained (23.8 g), which was pure enough for further use. A portion of this oil was purified by flash chromatography with ethyl acetate as eluent (R_f = 0.4). The ^1H NMR spectrum of the purified oil indicated complete removal of the protective group on the 2-position.

^1H NMR ($CDCl_3$): δ 1.35 (m, 8H, central $C\underline{H}_2$'s), 1.66 (m, 4H, 2*$C\underline{H}_2$), 2.71 (t, 2H, J = 8 Hz, imidazole-5-$C\underline{H}_2$), 2.88 (s, 6H, $N(C\underline{H}_3)_2$), 3.68 (t, 2H, J = 7 Hz, $C\underline{H}_2$-phthalimide), 6.82 (s, 1H, imidazole-4H), 7.75 (m, 5H, phthalimide-H + imidazole-2H) ppm.

1-(N,N-Dimethylsulfamoyl)-5-(10-phthalimidodecyl)imidazole (9h)

The same procedure as for the preparation of **9f** was employed, using the crude product **8h** (18.3 g) in 150 ml DMF and 9.3 g (50 mmol) potassium phthalimide. After work up a dark brown oil remained (11.2 g), which was pure enough for further use. A portion of this oil was purified by flash chromatography with ethyl acetate as eluent (R_f = 0.4). The ^1H NMR spectrum of the purified oil indicated complete removal of the protective group on the 2-position.

^{1}H NMR ($CDCl_3$): δ 1.31 (m, 12H, central $C\underline{H}_2$'s), 1.68 (m, 4H, 2*$C\underline{H}_2$), 2.71 (t, 2H, J = 8 Hz, imidazole-5-$C\underline{H}_2$), 2.88 (s, 6H, $N(C\underline{H}_3)_2$), 3.68 (t, 2H, J = 7 Hz, $C\underline{H}_2$-phthalimide), 6.83 (s, 1H, imidazole-4H), 7.78 (m, 5H, phthalimide-H + imidazole-2H) ppm.

*1-(*N,N-*Dimethylsulfamoyl)-5-(6-aminohexyl)imidazole (**10f**)*

0.7 g (1.7 mmol) Of **9f** was dissolved in 40 ml. warm ethanol. 0.3 ml. (6.2 mmol) hydrazine monohydrate was added and this solution was heated under reflux for 4 hours. After cooling of the solution to room temperature, a white precipitate formed and was removed by filtration. The filtrate was concentrated in vacuo and 0.4 g (84%) of a light oil was isolated.

^{1}H NMR ($CDCl_3$): δ 1.33 (m, 6H, central $C\underline{H}_2$'s + $N\underline{H}_2$), 1.60 (m, 4H, 2*$C\underline{H}_2$), 2.64 (m, 4H, imidazole-5-$C\underline{H}_2$ + $C\underline{H}_2NH_2$), 2.83 (s, 6H, $N(C\underline{H}_3)_2$), 6.75 (s, 1H, imidazole-4H), 7.78 (s, 1H, imidazole-2H) ppm.

*1-(*N,N-*Dimethylsulfamoyl)-5-(8-aminooctyl)imidazole (**10g**)*

7.6 g (17.6 mmol) Of **9g** was dissolved in 100 ml. warm ethanol. 2.5 ml. (51 mmol) hydrazine monohydrate was added and this solution was heated under reflux for 1 h. A white precipitate formed and was removed by filtration. The filtrate was concentrated in vacuo and 5.2 g (98 %) of a light oil was isolated.

^{1}H NMR ($CDCl_3$): δ 1.32 (m, 10H, central $C\underline{H}_2$'s + $N\underline{H}_2$), 1.62 (m, 4H, 2*$C\underline{H}_2$), 2.68 (m, 4H, imidazole-5-$C\underline{H}_2$ + $C\underline{H}_2NH_2$), 2.87 (s, 6H, $N(C\underline{H}_3)_2$), 6.72 (s, 1H, imidazole-4H), 7.84 (s, 1H, imidazole-2H) ppm.

*1-(*N,N-*Dimethylsulfamoyl)-5-(10-aminodecyl)imidazole (**10h**)*

The same procedure as for the preparation of **10g** was employed, using 2.0 g (4.4 mmol) of **9h** in 50 ml ethanol and 0.6 ml (14 mmol) hydrazine monohydrate. 1.2 g (84%) Of a light oil was isolated.

^{1}H NMR ($CDCl_3$): δ 1.32 (m, 14H, central $C\underline{H}_2$'s + $N\underline{H}_2$), 1.62 (m, 4H, 2*$C\underline{H}_2$, 2.66 (m, 4H, imidazole-5-$C\underline{H}_2$ + $C\underline{H}_2NH_2$), 2.87 (s, 6H, $N(C\underline{H}_3)_2$), 6.72 (s, 1H, imidazole-4H), 7.84 (s, 1H, imidazole-2H) ppm.

*4(5)-(6-Aminohexyl)-1H-imidazole dioxalate (**1f**)*

44.9 g Of **9f** was dissolved in 30 % HBr (500 ml) and heated under reflux. After 16 h the mixture was cooled, filtered and concentrated in vacuo. The residue was dissolved in absolute ethanol (300 ml), heated under reflux for 0.5 h and concentrated under reduced pressure. The remaining dark oil was washed (under stirring) with 100 ml portions of acetone (removal of the formed ethyl *N,N*-dimethylsulfamoate group). After several washings, the oil crystallized. After filtration of the brown solid, 17.6 g (66% overall) of the product was obtained.
The dihydrobromide was converted into the dioxalate, using the following procedure : the dihydrobromide was dissolved in absolute EtOH and 2 equiv of sodium ethanolate was added. This was refluxed for 0.5 h and after filtration of sodium bromide the filtrate was concentrated in vacuo. The residue was dissolved in *iso*-propanol, filtrated (removal of some remaining sodium bromide) and a saturable solution of oxalic acid in *iso*-propanol was added dropwise until a white precipitate was formed. The precipitate was collected by centrifugation and the pellet was washed three times with *iso*-propanol, dried and recrystallized from hot *iso*-propanol.
A white powder was collected. M.p. 132.0 °C.

^{1}H NMR (D_2O) : δ 1.33 (m, 4H, central $C\underline{H}_2$'s), 1.61 (m, 4H, 2*$C\underline{H}_2$), 2.68 (t, 2H, J=7 Hz, imidazole-4(5)-$C\underline{H}_2$), 2.93 (t, 2H, J=7 Hz, $C\underline{H}_2NH$), 7.14 (s, 1H, imidazole-5(4)H), 8.50 (s, 1H, imidazole-2H) ppm.

^{13}C NMR (D_2O) : δ 25.0, 26.7, 28.0, 28.9, 40.9, 116.5, 134.0, 135.4, 166.7.

MS (EI) : m/z 167 (M^+, 2%), 151 (M^+-NH_2, 6%), 137 ($[ImC_5H_{10}]^+$, 4%), 123 ($[ImC_4H_8]^+$, 3%), 109 ($[ImC_3H_6]^+$, 5%), 95 ($[ImC_2H_4]^+$, 39%), 82 ($[ImCH_3^+$, 31%), 81 ($[ImCH_2]^+$, 15%), 45 ($[C_2H_7N]^+$, 100%).

High resolution mass : m/z 167.1426; calcd. for $C_9H_{17}N_3$: 167.1422.

*4(5)-(8-Aminooctyl)-1H-imidazole dioxalate (**1g**)*

The same procedure as for the synthesis of **1f** was used with the exception that 2.3 g of **9g** was heated under reflux in 30% HBr (30 ml). After several washings with acetone, 1.14 g (67% overall) of a brown solid was collected. The dihydrobromide was converted into a dioxalate. A white powder was obtained. M.p. (95.0-97.0) °C.

^{1}H NMR (D_2O) : δ 1.38 (m, 8H, central C$\underline{H_2}$'s), 1.60 (m, 4H, 2*C$\underline{H_2}$), 2.66 (t, 2H, J=7 Hz, imidazole-4(5)-C$\underline{H_2}$), 2.93 (t, 2H, J=7 Hz, C$\underline{H_2}$NH), 7.13 (s, 1H, imidazole-5(4)H), 8.49 (s, 1H, imidazole-2H) ppm.

^{13}C NMR (D_2O) : δ 25.1, 27.0, 28.1, 29.0, 29.3, 29.49, 29.51, 41.0, 116.4, 133.8, 135.7, 165.6.

MS (EI) : m/z 195 (M^+, 0.6%), 179 (M^+-NH_2, 1%), 165 ([ImC_7H_{14}]$^+$, 1%), 151 ([ImC_6H_{12}]$^+$, 1%), 137 ([ImC_5H_{10}]$^+$, 2%), 123 ([ImC_4H_8]$^+$, 1%), 109 ([ImC_3H_6]$^+$, 2%), 95 ([ImC_2H_4]$^+$, 8%), 82 ([$ImCH_3$]$^+$, 9%), 81 ([$ImCH_2$]$^+$, 5%), 45 ([C_2H_7N]$^+$, 100%).

High resolution mass : m/z 195.1732; calcd. for $C_{11}H_{21}N_3$: 195.1736.

Anal. ($C_{11}H_{21}N_3$•2.15$C_2H_2O_4$) : 47.13 %C, 6.87 %H, 10.9 %N;
calcd. : 47.26 %C, 6.56 %H, 10.8 %N.

*4(5)-(10-Aminodecyl)-1*H*-imidazole dioxalate (**1h**)*

The same procedure as for the synthesis of **1f** was used with the exception that 2.2 g of **9h** was heated under reflux in 30% HBr (30 ml). After several washings with acetone, 0.6 g (27% overall) of a brown solid was collected. The dihydrobromide was converted into a dioxalate. A white powder was obtained. M.p. (154.5-155.0) °C.

^{1}H NMR (D_2O) : δ 1.27 (m, 12H, central C$\underline{H_2}$'s), 1.62 (m, 4H, 2*C$\underline{H_2}$), 2.67 (t, 2H, J=7 Hz, imidazole-4(5)-C$\underline{H_2}$), 2.94 (t, 2H, J=7 Hz, C$\underline{H_2}$NH), 7.14 (s, 1H, imidazole-5(4)H), 8.50 (s, 1H, imidazole-2H) ppm.

^{13}C NMR (D_2O) : δ 25.1, 27.0, 28.2, 29.1, 29.4, 29.6, 29.7, 29.88, 29.93, 41.0, 116.4, 133.8, 135.7, 167.3.

MS (EI) : m/z 223 (M^+, 3%), 207 (M^+-NH_2, 4%), 193 ([ImC_9H_{18}]$^+$, 5%), 179 ([ImC_8H_{16}]$^+$, 6%), 165 ([ImC_7H_{14}]$^+$, 5%), 151 ([ImC_6H_{12}]$^+$, 6%), 137 ([ImC_5H_{10}]$^+$, 6%), 123 ([ImC_4H_8]$^+$, 3%), 109 ([ImC_3H_6]$^+$, 6%), 95 ([ImC_2H_4]$^+$, 27%), 82 ([$ImCH_3$]$^+$, 26%), 81 ([$ImCH_2$]$^+$, 13%), 45 ([C_2H_7N]$^+$, 100%).

High resolution mass : m/z 223.2049; calcd. for $C_{13}H_{25}N_3$: 223.2048.

Anal. ($C_{13}H_{25}N_3$•1.7$C_2H_2O_4$) : 52.15 %C, 7.62 %H, 11.3 %N;
calcd. : 52.33 %C, 7.60 %H, 11.2 %N.

Pharmacology

The histamine H_3 activity of the histamine homologues (**1**), was determined on an *in vitro* assay, on the basis of the inhibitory effect of histamine H_3 agonists on electrically evoked twitches (induced by endogenous acetylcholine release) of guinea pig jejunum preparations (see Chapter 3).[15] The addition of cumulative concentrations of an H_3 agonist, results in a concentration-dependent inhibition of these evoked twitches, from which a concentration-response curve can be constructed. All histamine homologues (**1**) were tested for H_3 agonism and antagonism. H_3 antagonism was determined against (R)-α-methylhistamine. The potency of the antagonists was expressed by its pA_2 value, calculated from the Schild regression analysis, and at least three different concentrations were used. Statistical analysis was carried out with the Students' *t*-test and $p < 0.05$ was considered statistically significant. Each compound was tested on tissue preparations of at least four different animals in triplicate.

The binding affinity of the described compounds for the H_1 receptor was determined in at least three independent experiments, performed in triplicate, by the displacement of [^{3}H]mepyramine, bound to membranes of CHO cells expressing guinea pig H_1 receptors.[122]

The binding affinity of the described compounds for the H_2 receptor was established in at least three independent experiments, performed in triplicate, by the displacement of [^{125}I]iodoaminopotentidine, bound to membranes of CHO cells expressing human H_2 receptors.[123]

Acknowledgement. We thank Dr. B. van Baar for the determination of the MS data. The research of Dr. R. Leurs has been made possible by a fellowship of the Royal Netherlands Academy of Arts and Sciences.

Chapter 8

Analogues of Burimamide as Potent and Selective Histamine H_3 Receptor Antagonists

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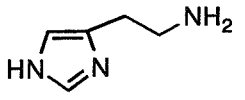
Abstract

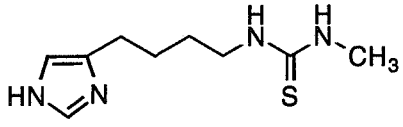
Burimamide was one of the first compounds reported to antagonize the activation of the histamine H_3 receptor by histamine. We have prepared a large series of burimamide analogues by variation of the alkyl spacer length of burimamide from two methylene groups to six methylene groups and also by replacement of the *N*-methyl group with other alkyl and aryl groups. All analogues are reversible competitive H_3 antagonists as determined on the guinea pig intestine. Elongation of the alkyl chain from an ethylene chain to a hexylene chain, results in an increase of the H_3 antagonistic activity. The H_3 selective pentylene and the hexylene analogues of burimamide are about ten times more potent than burimamide. The *N*-thiourea substituents however, have no beneficial influence on the affinity.

Introduction

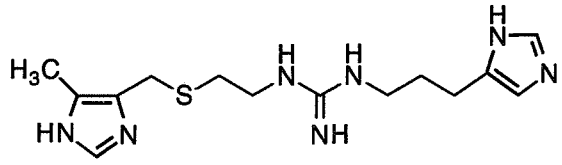
The existence of a third histamine receptor subtype, inhibiting the synthesis and release of histamine, located presynaptically in histaminergic nerve endings in rat cerebral cortex, was suggested in 1983 by Arrang *et al.*[5] Confirmation of the existence of this new histamine receptor subtype was provided by the development of the H_3 selective agonist (R)-α-methylhistamine and the H_3 selective antagonist thioperamide.[6] The H_3 receptor has since been shown to play an important regulatory role in the release of other neurotransmitters in the central nervous system[7-10] and in the periphery.[11-16]



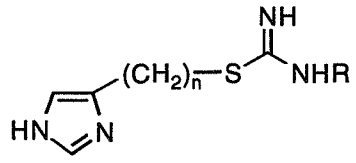
Histamine; **1.2**



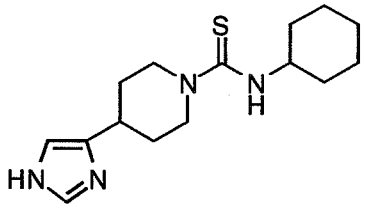
Burimamide; **3.4a**



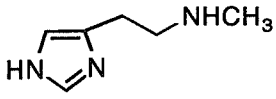
Impromidine



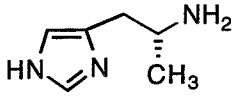
Imetit ; n=2, R=H
VUF 8621 ; n=2, R=me
VUF 8328 ; n=3, R=H
Clobenpropit ; n=3, R=4-chlorobenzyl



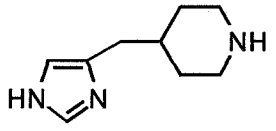
Thioperamide



N^{α}-Methylhistamine



(R)-α-Methylhistamine



Immepip

Figure 1. Discussed structures.

A few years before the identification of the H_3 receptor, the antagonistic effect of the H_2 antagonist burimamide on the inhibitory action of histamine on electrically evoked contractions of guinea pig intestine preparations was described.[74] This inhibitory effect of histamine was reversible and not mediated by adrenergic- nor H_1 receptors.[73] The histamine H_2 antagonist burimamide was able to block this inhibitory effect of histamine, but the insensitivity of the evoked contractions to H_2 agonists made it doubtful that this effect was mediated by the H_2 receptor. Further evidence for the distinct difference between the "classical" H_2 receptors in the heart and these histamine-stimulated, contraction-inhibiting receptors on the guinea pig ileum was given by Fjalland *et al.*[75] The antagonistic effect of burimamide on the inhibitory guinea pig ileum receptor was described to be about 25 times higher than that of another H_2 antagonist, cimetidine, whereas on the H_2 receptor in the heart, cimetidine was described to be at least 10 times more potent as an H_2 antagonist than burimamide.

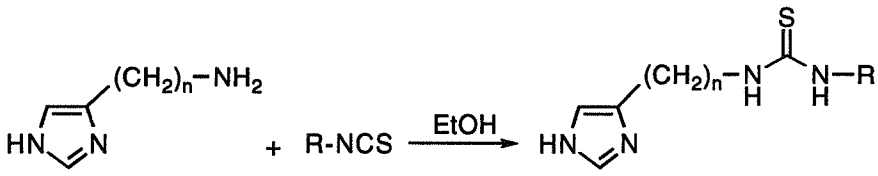
After the discovery of the histamine H_3 receptor and the description of the antagonistic effect of burimamide on this receptor, the inhibitory histamine receptor on the guinea pig intestine was suggested to be of the H_3 subtype as well.[76] Burimamide was therefore one of the first compounds discovered, to antagonize the H_3 receptor and played a major role in its elucidation. The compounds' lack of selectivity however, makes it less attractive as a pharmacological tool for this receptor.

The first potent and selective antagonist for the histamine H_3 receptor was thioperamide, as derived from a series of rigid analogues of histamine.[6] This compound possesses several distinct structural features, which are also present in the structure of burimamide; an *N*-alkyl substituted thiourea group and an alkyl spacer on the 4(5)-position of an imidazole ring. The cyclohexyl group in the structure of thioperamide has been reported to be optimal for a high affinity on the H_3 receptor.[48]

Thioperamide can be seen as a rigid analogue of burimamide, but is more potent and selective as an H_3 antagonist. Two important differences in the structure of burimamide and thioperamide are the length of the alkyl spacer between the imidazole and the thiourea group (a butylene chain in the structure of burimamide and a propylene chain in the structure of thioperamide), and the *N*-alkyl substituent on the thiourea group (a methyl group for burimamide and a cyclohexyl group for thioperamide).

This raises the question if burimamide has the optimal structure for its H_3 antagonistic properties and whether the antagonistic activity and its selectivity for the H_3 receptor can be increased with some structural modifications. Not many structural variations of burimamide and their activity on the histamine H_3 receptor are known. A strong influence of the chain length of the alkyl spacer of burimamide on the H_3 activity has been demonstrated, since a burimamide analogue with a propylene chain (norburimamide) is only a weak antagonist, with a pA_2 value of 6.1 for the H_3 receptor, compared to a pA_2 value of 7.2 of burimamide (both on rat cortex).[54]

We wanted to study the influence of the chain length of the alkyl spacer in the structure of burimamide derivatives on the H_3 activity. We additionally wished to evaluate the influence of the *N*-thiourea substituents on the activity of this receptor. Therefore we have prepared a large series of analogues of burimamide and determined the H_3 activity of these compounds functionally on an *in vitro* test system using guinea pig jejunum preparations.[15] In this series we varied the length of the alkyl spacer of burimamide from two to six methylene groups and additionally replaced the methyl group by other alkyl and aryl groups. We investigated the selectivity of the most potent analogues as well, by determining their affinity for the H_1 and the H_2 receptors.



1 **2a-i** **3**

1.2; n=2
1.3; n=3
1.4; n=4
1.5; n=5
1.6; n=6

a; R=methyl
b; R=ethyl
c; R=*n*-propyl
d; R=*i*-propyl
e; R=cyclohexyl
f ; R=phenyl
g; R=benzyl
h; R=phenylethyl
i ; R=4-chlorobenzyl

3.2 a-h ; n=2
3.3 a-h ; n=3
3.4 a-h ; n=4
3.5 a-i ; n=5
3.6 a, f ; n=6

Scheme 1. Synthesis of the burimamide analogues **3** from 4(5)-(ω-aminoalkyl)-1*H*-imidazoles **1**.

Chemistry

The burimamide analogues were prepared by reaction of the corresponding 4(5)-(ω-aminoalkyl)-1*H*-imidazoles with a series of alkyl- cq. aryl isothiocyanates (see scheme 1). The 4(5)-(ω-aminoalkyl)-1*H*-imidazoles **1.3-1.6**, were prepared using a method described earlier by our group.[121] All isothiocyanates were commercially available. Most of the compounds were isolated as oxalates, because of better stability and isolation.

Pharmacology

The H_3 activity of the compounds was determined on an *in vitro* test system, on the basis of the concentration-dependent inhibitory effect of histamine H_3 agonists, on the electrically evoked contractile response of isolated guinea pig jejunum segments.[15]

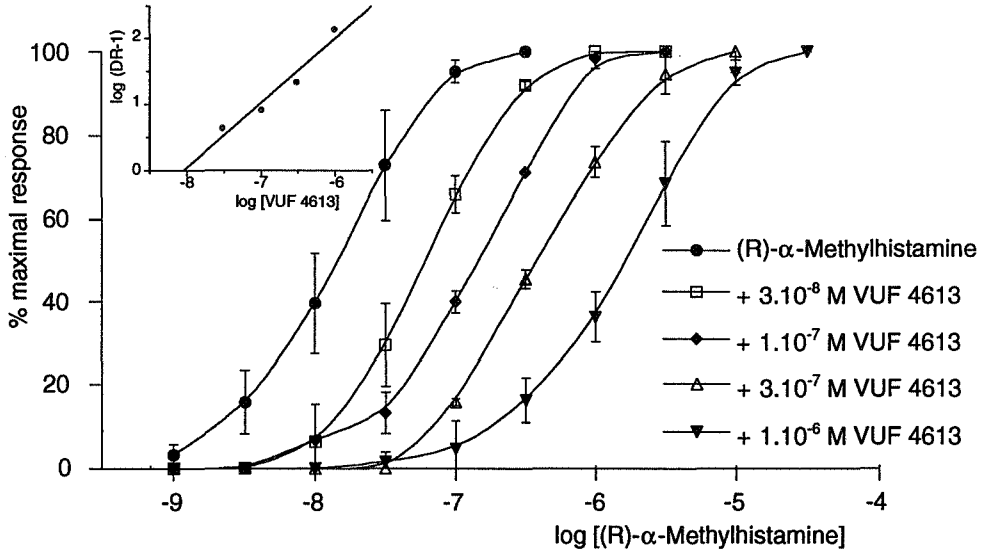


Figure 2. Concentration-response curves of (R)-α-methylhistamine, with a rightward parallel shift upon addition of VUF 4613 (corrected to 100%). The Schild plot of these results is shown in the inset.

The affinity of the selected compounds for the H_1 receptor was determined by the displacement of [^{3}H]mepyramine, bound to membranes of CHO cells expressing guinea pig H_1 receptors.[122] The affinity of the selected compounds for the H_2 receptor was established by displacement of [^{125}I]iodoaminopotentidine, bound to membranes of CHO cells expressing human H_2 receptors.[123]

Results and Discussion

All the synthesized analogues of burimamide are reversible competitive antagonists on the histamine H_3 receptor, as determined on guinea pig jejunum, with Schild slopes not significantly different from unity (see table 1).

The burimamide analogues **2.2a-2.2h**, with an ethylene chain, which can be seen as derivatives of histamine, are only weak H_3 antagonists. This means that replacement of the positively charged, ammonium group (at physiological pH) of histamine, by a neutral *N*-substituted thiourea group, results in loss of intrinsic activity on the H_3 receptor. This might be due to steric hindrance, since N^{α}-methylhistamine is a potent agonist for the H_3 receptor, and replacement of the *N*-methyl group by a propyl group results in a compound without H_3 activity.[54] Moreover the reduced affinity might be the result of the altered electronic properties. Most of the described potent H_3 agonists so far are compounds with an imidazole ring and an amino group (e.g., (R)-α-methylhistamine and immepip), separated by an alkyl spacer.

The imidazole ring seems to be essential for activation, since replacement of the imidazole ring by other heterocyclic rings, resulted in less active compounds, or compounds deprived of any agonistic activity.[26,41] The amino group of histamine however, which is protonated at physiological pH, has been replaced with other basic groups, like an isothiourea group, resulting in potent H_3 agonists (e.g., imetit).[37-40] The pK_a of the isothiourea group (pK_a = 9-10) has been described to be similar to aliphatic amines (pK_a = 9-11).[40] Monomethylation of the isothiouronium moiety in imetit does not drastically affect the agonistic activity on the H_3 receptor (pD_2 value of VUF 8621 is 7.3, compared to a pD_2 value of 8.1 of imetit on the guinea pig ileum),[13,37,40] whereas the ethylene derivative of burimamide, VUF 4577 (**3.2a**) is a weak H_3 antagonist. Because the thiourea group of VUF 4577 (**3.2a**) is uncharged at physiological pH, it seems that a specific ionic binding site at the H_3 receptor for cationic groups of H_3 agonists, probably a carboxylate (e.g., an aspartate residue), exists.

Table 1. Histamine H_3 antagonistic activity of the burimamide analogues **3** as determined on the *in vitro* test system on the guinea pig jejunum.

Compound	VUF[a]	n[b]	R[c]	pA_2[d]	Slope[e]	N[f]
3.2a	4577	2	methyl	5.5 ± 0.2	1.0 ± 0.1	3
3.2b	4578	2	ethyl	5.3 ± 0.2	1.1 ± 0.2	4
3.2c	4579	2	*n*-propyl	5.4 ± 0.2	1.0 ± 0.1	4
3.2d	4580	2	*i*-propyl	4.8 ± 0.1	0.9 ± 0.1	3
3.2e	4581	2	cyclohexyl	5.9 ± 0.2	1.1 ± 0.1	3
3.2f	4582	2	phenyl	5.2 ± 0.2	1.0 ± 0.1	3
3.2g	4583	2	benzyl	5.8 ± 0.2	1.1 ± 0.2	3
3.2h	4584	2	phenylethyl	5.9 ± 0.1	1.0 ± 0.1	3
3.3a	4630	3	methyl	6.4 ± 0.2	1.0 ± 0.1	4
3.3b	4631	3	ethyl	7.1 ± 0.2	1.0 ± 0.1	4
3.3c	4632	3	*n*-propyl	7.0 ± 0.2	1.2 ± 0.1	4
3.3d	4633	3	*i*-propyl	7.1 ± 0.2	1.0 ± 0.1	4
3.3e	4634	3	cyclohexyl	6.9 ± 0.2	1.1 ± 0.1	4
3.3f	4635	3	phenyl	6.9 ± 0.1	1.1 ± 0.1	4
3.3g	4636	3	benzyl	6.7 ± 0.2	1.1 ± 0.1	4
3.3h	4637	3	phenylethyl	6.7 ± 0.2	1.1 ± 0.1	4
3.4a	4680	4	methyl	7.0 ± 0.2	1.0 ± 0.1	5
3.4b	4681	4	ethyl	7.4 ± 0.2	1.1 ± 0.2	4
3.4c	4682	4	*n*-propyl	7.3 ± 0.3	1.2 ± 0.3	4
3.4d	4683	4	*i*-propyl	7.5 ± 0.1	1.0 ± 0.3	4
3.4e	4684	4	cyclohexyl	7.1 ± 0.2	1.1 ± 0.3	4
3.4f	4685	4	phenyl	7.6 ± 0.2	1.0 ± 0.3	4
3.4g	4686	4	benzyl	7.1 ± 0.3	1.2 ± 0.3	4
3.4h	4687	4	phenylethyl	7.0 ± 0.2	1.3 ± 0.1	3

Table 1. (continued)

Compound	VUF[a]	n[b]	R[c]	pA_2[d]	Slope[e]	N[f]
3.5a	4613	5	methyl	8.0 ± 0.1	1.0 ± 0.1	3
3.5b	4614	5	ethyl	8.0 ± 0.1	1.0 ± 0.1	4
3.5c	4615	5	*n*-propyl	7.7 ± 0.1	1.2 ± 0.1	4
3.5d	4616	5	*i*-propyl	7.7 ± 0.1	1.2 ± 0.1	4
3.5e	4617	5	cyclohexyl	7.5 ± 0.1	1.0 ± 0.1	4
3.5f	4618	5	phenyl	7.6 ± 0.2	1.0 ± 0.2	3
3.5g	4619	5	benzyl	7.7 ± 0.2	1.0 ± 0.1	3
3.5h	4620	5	phenylethyl	7.5 ± 0.2	1.1 ± 0.2	3
3.5i	4742	5	4-Cl-benzyl	8.1 ± 0.2	0.9 ± 0.1	3
3.6a	4740	6	methyl	7.9 ± 0.1	1.0 ± 0.1	5
3.6f	4741	6	phenyl	8.0 ± 0.2	0.9 ± 0.2	3

[a] Compound code number.
[b] Alkyl chain length of **3** (number of methylene units).
[c] Substituent of **3**.
[d] Antagonistic parameter as determined on the described *in vitro* H_3 assay representing the negative logarithm of the abscissal intercept from the Schild plot ± SD.
[e] Slope of Schild plot ± SD, not significantly different from unity.
[f] Number of different animal preparations.

Elongation of the alkyl chain of the burimamide analogues from a propylene chain to a hexylene chain, results in an increase of the H_3 antagonistic activity. The pentylene chain seems to be optimal in length for H_3 antagonistic activity for these analogues. Replacement of the pentylene chain of VUF 4613 (**3.5a**) for instance, by a hexylene chain, does not lead to increased H_3 activity (VUF 4740 (**3.6a**)).

Table 2. Selectivity of the pentylene analogues of burimamide **3.5a-3.5h** for the H_3 receptor.

Comp	VUF[a]	n[b]	R[c]	pK_i H_1[d]	pK_i H_2[e]	pA_2 H_3[f]
3.5a	4613	5	methyl	4.7±0.1	4.7±0.1	8.0 ± 0.1
3.5b	4614	5	ethyl	4.8±0.1	5.0±0.1	8.0 ± 0.1
3.5c	4615	5	*n*-propyl	5.5±0.1	5.3±0.1	7.7 ± 0.1
3.5d	4616	5	*i*-propyl	4.9±0.1	5.0±0.1	7.7 ± 0.1
3.5e	4617	5	cyclohexyl	5.1±0.1	5.4±0.1	7.5 ± 0.1
3.5f	4618	5	phenyl	5.6±0.1	4.9±0.1	7.6 ± 0.2
3.5g	4619	5	benzyl	5.4±0.1	5.8±0.2	7.7 ± 0.2
3.5h	4620	5	phenylethyl	5.5±0.1	5.5±0.3	7.5 ± 0.2
3.5i	4742	5	4-Cl-benzyl	5.8±0.1	5.8±0.2	8.1 ± 0.2

[a] Compound code number.
[b] Alkyl chain length of **3** (number of methylene units).
[c] Substituent of **3**.
[d] Log value of the binding affinity for the histamine H_1 receptor ± SEM.
[e] Log value of the binding affinity for the histamine H_2 receptor ± SEM.
[f] Antagonistic parameter as determined on the described *in vitro* H_3 assay representing the negative logarithm of the abscissal intercept from the Schild plot ± SD.

The affinity for the H_1- and the H_2 receptor is determined for these potent pentylene analogues (**3.5a-3.5i**) (see Table 2). Clearly these compounds are selective for the H_3 receptor, although the *N*-methyl substituted pentylene analogue, VUF 4613 (**3.5a**) is more selective than the more lipophilic *N*-4-chlorobenzyl substituted analogue VUF 4742 (**3.5i**). This highly selective pentylene analogue of burimamide VUF 4613 (**3.5a**), is ten times more potent than burimamide.

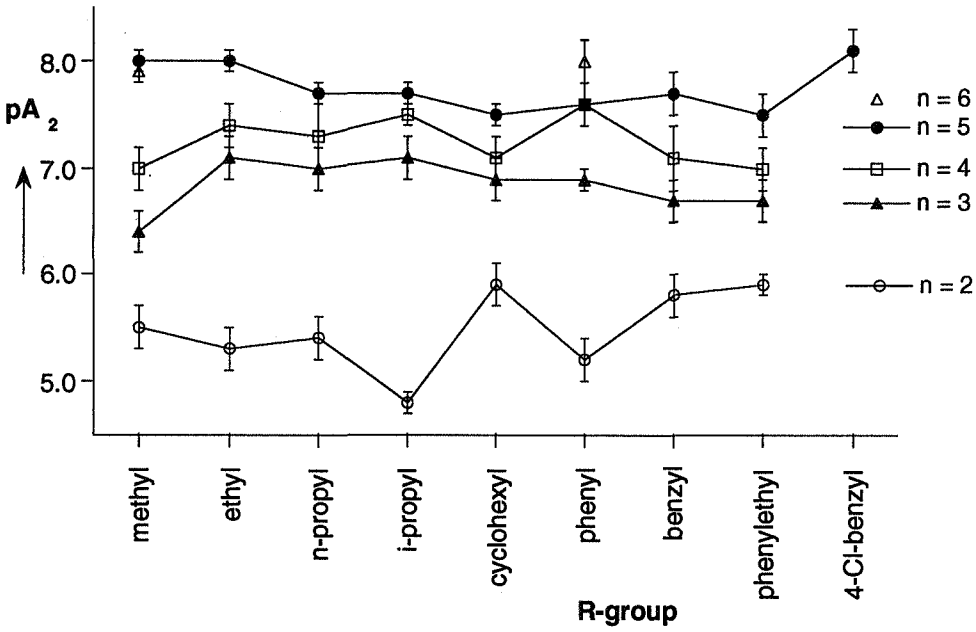


Figure 3. The influence of the alkyl chain length (n) and the *N*-thiourea substituent (R) of the burimamide analogues **3** on the pA_2 value on the histamine H_3 receptor. Lines have been drawn for easy recognition of these influences.

The large influence of the length of the alkyl spacer, on the H_3 activity of the burimamide analogues, is clearly visible in Figure 3. From this figure, the lack of influence of the *N*-thiourea substituents on the H_3 activity however, is also apparent. If we consider the analogues with a pentyl chain (n=5), there is not a great difference in pA_2 value between the compounds containing a small alkyl group, a large alkyl group or an aromatic substituent.

This suggests that the receptor binding of this part of the burimamide analogues is not through a hydrophobic interaction, nor through an electrostatic π–π interaction between aromatic systems. These results are rather surprising, since it has been proposed that an H_3 antagonist should consist of an *N*-containing heterocycle, linked to a polar group by an alkyl chain, with a lipophilic residue attached to the polar group, for enhancement of the affinity.[26]

A clear example of the affinity enhancing effect of lipophilic residues can be observed in the series of analogues of imetit, as described by Van der Goot *et al.*[37] In this series, derivatization of the potent H_3 antagonist VUF 8328 (pA_2 value of 8.0 on

guinea pig ileum), leads to compounds with even higher affinity for the H_3 receptor. The introduction of a *p*-chlorobenzyl group on the isothiourea group of VUF 8328, resulted in the most potent H_3 antagonist described so far (clobenpropit), with a pA_2 value of 9.9 on the guinea pig ileum.
The introduction of lipophilic residues on the thiourea group of the burimamide analogues however, does not enhance the H_3 antagonistic activity. This seems to rule out a possible interaction of this series of antagonists in the same manner as the isothiourea derivatives of Van der Goot.[24]
Thioperamide also binds in a distinct manner to the H_3 receptor, other than the burimamide analogues, since VUF 4634 (**3.3e**) is about hundred times less potent, as an H_3 antagonist, than thioperamide, which can be seen as its rigid analogue. Because there is no large influence of the *N*-thiourea substituents of the burimamide analogues on the pA_2 value, only an interaction of the thiourea group with the receptor via hydrogen bonding seems likely.

It can be concluded that the intrinsic activity of histamine on the H_3 receptor is lost when the amino group is replaced by an *N*-substituted thiourea group. Elongation of the alkyl spacer leads to an increase of affinity, with a pentylene chain being optimal, indicating an additional binding site for the pentylene and higher analogues of burimamide. Burimamide is not the optimal antagonist in this series. The chain length of the alkyl spacer has a large influence on the H_3 antagonistic activity, with VUF 4613 being ten times more potent than burimamide. The *N*-thiourea substituents however, have no great influence on the affinity. These results indicate a binding behaviour for the burimamide analogues in a non-lipophilic environment, different from other H_3 antagonists like thioperamide and clobenpropit. Although burimamide was originally described as an H_2 antagonist, the more potent pentylene analogues of burimamide are highly selective for the H_3 receptor.

Experimental Section

Chemistry.

^1H NMR spectra were recorded on a Bruker AC-200 (200 MHz) spectrometer with tetramethylsilane or sodium 3-(trimethylsilyl)propionate as an internal standard. Mass spectra were recorded on a Finnigan MAT-90. Melting points were measured on a Mettler FP-5 + FP-52 apparatus and are uncorrected. Histamine dihydrochlo-

ride (**1.2**) was purchased from Janssen Chimica. 4(5)-(3-Aminopropyl)-1*H*-imidazole dihydrobromide (**1.3**), 4(5)-(4-aminobutyl)-1*H*-imidazole dihydrobromide (**1.4**) and 4(5)-(5-aminopentyl)-1*H*-imidazole dihydrobromide (**1.5**) were prepared as described earlier by our group.[121] 4(5)-(6-aminohexyl)-1*H*-imidazole (**1.6**) was prepared using the same method.[121,131] Methyl-, (**2a**) and ethyl isothiocyanate (**2b**) were purchased from Aldrich; *n*-propyl-, (**2c**) *i*-propyl-, (**2d**) benzyl-, (**2g**) and phenylethyl isothiocyanate (**2h**) from Maybridge Chemical Co. (MCC); Cyclohexyl-, (**2e**) and phenyl isothiocyanate (**2f**) from Janssen Chimica and chlorobenzyl isothiocyanate (**2i**) was purchased from Lancaster. The isothiocyanates were used without purification. The purity of the products was checked on thin layer chromatography (Merck silica gel 60, F254, 0.25mm). The free bases of all compounds gave one spot using either ethyl acetate ($R_f \approx 0$-0.1), methanol ($R_f \approx 0.9$-1.0) or $CHCl_3$ ($R_f \approx 0.5$). The yields of the purified salt are given.

General Procedure

The required 4(5)-(ω-aminoalkyl)-1*H*-imidazole (**1.2-1.6**) either as dihydrochloride or as dihydrobromide, was added to 2 equivalent sodium ethanolate in absolute ethanol. This solution was refluxed for 30 min., and cooled to room temperature. The formed precipitate was removed by filtration and 3 equivalent of the needed isothiocyanate was added to the filtrate. The ethanol was removed under reduced pressure, after 2 h refluxing. The residue was purified by column-chromatography, by washing with ethyl acetate as eluent (isothiocyanate eluted $R_f = 1.0$). The product was subsequently eluted with methanol as eluent (unreacted amine remained on column). After removal of the methanol under reduced pressure, the free base was converted into a hydrobromide or an oxalate.

The hydrobromides were prepared by solvation of the free base in 10% HBr-solution. After stirring at room temperature for 15 min, the acidic solution was concentrated *in vacuo*, triturated three times with absolute ethanol and recrystallized from ethanol/ethyl acetate.

The oxalates were prepared by solvation of the free base in ethyl acetate and the addition of an excess of a saturated solution of oxalic acid in ethyl acetate (slowly). The formed precipitate was collected by centrifugation, washed with ethyl acetate (three times) and recrystallized from absolute ethanol.

N-*Methyl*-N'-*[2-(4(5)-imidazolyl)ethyl]thiourea hydrobromide (VUF 4577)* ***(3.2a)***

Melting point 99.9-100.8°C. Yield 49%.

^{1}H NMR (D_2O) : δ 2.87 (s, 3H, C<u>H</u>$_3$), 3.03 (t, 2H, J = 7 Hz, imidazole-C<u>H</u>$_2$), 3.78 (t, 2H, J = 7 Hz, C<u>H</u>$_2$NH), 7.30 (s, 1H, imidazole-5(4)H), 8.62 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 184 (M^+, 57%), 153 (M^+-CH_3NH_2, 47%), 150 (M^+-H_2S, 54%), 95 ($[ImC_2H_4]^+$, 100%), 81 ($[ImCH_2]^+$, 84%).

High resolution mass : m/z 184.0782; calcd. for $C_7H_{12}N_4S$: 184.0783.

Anal. ($C_7H_{12}N_4S$•2HBr)	: 24.33 %C, 4.19 %H, 16.16 %N;
Calcd.	: 24.29 %C, 4.08 %H, 16.19 %N.

N-*Ethyl*-N'-*[2-(4(5)-imidazolyl)ethyl]thiourea hydrobromide (VUF 4578)* ***(3.2b)***

Melting point 164.5-165.0°C. Yield 74%.

^{1}H NMR (D_2O) : δ 1.12 (t, 3H, J = 7 Hz, C<u>H</u>$_3$), 3.03 (t, 2H, J = 7 Hz, imidazole-C<u>H</u>$_2$), 3.32(q, 2H, J = 7 Hz, C<u>H</u>$_2$CH$_3$), 3.78 (t, 2H, J = 7 Hz, C<u>H</u>$_2$NH), 7.39 (s, 1H, imidazole-5(4)H), 8.62 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 198 (M^+, 50%), 164 (M^+-H_2S, 32%), 153 (M^+-$C_2H_5NH_2$, 18%), 95 ($[ImC_2H_4]^+$, 100%), 81 ($[ImCH_2]^+$, 51%).

High resolution mass : m/z 198.0940; calcd. for $C_8H_{14}N_4S$: 198.0939.

Anal. ($C_8H_{14}N_4S$•1.96HBr)	: 26.94 %C, 4.65 %H, 15.78 %N;
Calcd.	: 26.93 %C, 4.51 %H, 15.70 %N.

N-(n-*Propyl*)-N'-*[2-(4(5)-imidazolyl)ethyl]thiourea hydrobromide (VUF 4579)* ***(3.2c)***

Melting point 172.6-173.1°C. Yield 36%.

^{1}H NMR (D_2O) : δ 0.88 (t, 3H, J = 7 Hz, C<u>H</u>$_3$), 1.53 (m, 2H, C<u>H</u>$_2$CH$_3$), 3.04 (t, 2H, J = 7 Hz, imidazole-C<u>H</u>$_2$), 3.10-3.45 (m, 2H, C<u>H</u>$_2$CH$_2$CH$_3$), 3.70-3.92 (m, 2H, C<u>H</u>$_2$NH), 7.30 (s, 1H, imidazole-5(4)H), 8.64 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 212 (M^+, 62%), 178 (M^+-H_2S, 5%), 153 (M^+-$C_3H_7NH_2$, 13%), 95 ($[ImC_2H_4]^+$, 100%), 81 ($[ImCH_2]^+$, 35%).

High resolution mass : m/z 212.1100; calcd. for $C_9H_{16}N_4S$: 212.1096.

Anal. ($C_9H_{16}N_4S$•HBr)	: 37.10 %C, 6.00 %H, 19.31 %N;
Calcd.	: 36.87 %C, 5.84 %H, 19.11 %N.

N-(iso-*Propyl*)-N'-*[2-(4(5)-imidazolyl)ethyl]thiourea oxalate (VUF 4580)* ***(3.2d)***

Melting point 123.1°C. Yield 53%.

^{1}H NMR (D_2O) : δ 1.01 (d, 6H, J = 7 Hz, 2*C$\underline{H}_3$), 2.90 (t, 2H, J = 7 Hz, imidazole-C$\underline{H}_2$), 3.58-3.75 (m, 2H, C$\underline{H}_2$NH), 3.75-4.10 (b s, 1H, C$\underline{H}$), 7.16 (s, 1H, imidazole-5(4)H), 8.49 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 212 (M^+, 59%), 178 (M^+-H_2S, 12%), 153 (M^+-$C_3H_7NH_2$, 19%), 95 ([ImC_2H_4]$^+$, 100%), 81 ([ImCH_2]$^+$, 52%).

High resolution mass : m/z 212.1090; calcd. for $C_9H_{16}N_4S$: 212.1096.

Anal. ($C_9H_{16}N_4S$•$C_2H_2O_4$)	: 43.69 %C, 6.08 %H, 18.70 %N;
Calcd.	: 43.70 %C, 6.00 %H, 18.53 %N.

N-*Cyclohexyl*-N'-*[2-(4(5)-imidazolyl)ethyl]thiourea oxalate (VUF 4581)* ***(3.2e)***

Melting point 161.7°C. Yield 92%.

^{1}H NMR (D_2O) : δ 0.99-1.85 (m, 10H, cyclohexyl-C$\underline{H}_2$'s), 2.97 (t, 2H, J = 7 Hz, imidazole-C$\underline{H}_2$), 3.50-3.90 (m, 3H, C$\underline{H}$ + C$\underline{H}_2$NH), 7.22 (s, 1H, imidazole-5(4)H), 8.53 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 252 (M^+, 57%), 218 (M^+-H_2S, 12%), 153 (M^+-$C_6H_{11}NH_2$, 30%), 95 ([ImC_2H_4]$^+$, 100%), 81 ([ImCH_2]$^+$, 72%).

High resolution mass : m/z 252.1401; calcd. for $C_{12}H_{20}N_4S$: 252.1409.

Anal. ($C_{12}H_{20}N_4S$•0.5$C_2H_2O_4$)	: 52.37 %C, 7.23 %H, 18.79 %N;
Calcd.	: 52.50 %C, 7.12 %H, 18.84 %N.

N-*Phenyl*-N'-*[2-(4(5)-imidazolyl)ethyl]thiourea hydrobromide (VUF 4582)* ***(3.2f)***

Melting point 148.6-148.9°C. Yield 74%.

^{1}H NMR (D_2O) : δ 2.94-3.03 (m, 2H, imidazole-C$\underline{H}_2$), 3.75-3.97 (m, 2H, C$\underline{H}_2$NH), 7.11-7.57 (m, 6H, phenyl-H + imidazole-5(4)H), 8.61 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 246 (M^+, 3%), 212 (M^+-H_2S, 7%), 153 (M^+-$C_6H_5NH_2$, 41%), 135 ([C_6H_5NCS]$^+$, 100%), 93 ([$C_6H_5NH_2$]$^+$, 62%), 95 ([ImC_2H_4]$^+$, 12%), 81 ([ImCH_2]$^+$, 72%), 77 ([C_6H_5]$^+$, 51%).

High resolution mass : m/z 246.0931; calcd. for $C_{12}H_{14}N_4S$: 246.0939.

Anal. ($C_{12}H_{14}N_4S$•HBr) : 44.11 %C, 4.66 %H, 17.24 %N;
Calcd. : 44.04 %C, 4.62 %H, 17.12 %N.

N-*Benzyl*-N'-*[2-(4(5)-imidazolyl)ethyl]thiourea oxalate (VUF 4583) (**3.2g**)*

Melting point 153.7-155.0°C. Yield 18%.

^{1}H NMR (DMSO-d_6) : δ 2.89 (t, 2H, J = 7 Hz, imidazole-C$\underline{H}_2$), 3.59-3.83 (m, 2H, C$\underline{H}_2$NH), 4.53-4.77 (m, 2H, C$\underline{H}_2$-phenyl), 7.18-7.38 (m, 6H, phenyl-H + imidazole-4(5)H), 7.85-8.00 (m, 1H, N$\underline{H}$), 8.72 (t, 1H, J = 6 Hz, N$\underline{H}$), 8.72 (s, 1H, imidazole-2H), 11.15-11.85 (m, NH + oxalate) ppm.

MS (EI) : m/z 260 (M^+, 28%), 226 (M^+-H_2S, 8%), 153 (M^+-$C_7H_7NH_2$, 20%), 95 ([ImC_2H_4]$^+$, 44%), 91 ([C_7H_7]$^+$, 100%), 81 ([ImCH_2]$^+$, 38%).

High resolution mass : m/z 260.1101; calcd. for $C_{13}H_{16}N_4S$: 260.1096.

Anal. ($C_{13}H_{16}N_4S$•$C_2H_2O_4$) : 51.12 %C, 5.27 %H, 16.11 %N;
Calcd. : 51.42 %C, 5.18 %H, 15.99 %N.

N-*(2-Phenylethyl)*-N'-*[2-(4(5)-imidazolyl)ethyl]thiourea oxalate (VUF 4584) (**3.2h**)*

Melting point 145.1-145.5°C. Yield 18%.

^{1}H NMR (D_2O) : δ 2.66-2.91 (m, 4H, imidazole-C$\underline{H}_2$ + C$\underline{H}_2$-phenyl), 3.32-3.80 (m, 4H, 2*C$\underline{H}_2$NH), 7.15 (s, 1H, imidazole-5(4)H), 7.10-7.34 (m, 5H, phenyl-H), 8.47 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 274 (M^+, 39%), 220 (M^+-H_2S, 2%), 153 (M^+-$C_8H_9NH_2$, 22%), 105 ([C_8H_9]$^+$, 42%), 95 ([ImC_2H_4]$^+$, 100%), 91 ([C_7H_7]$^+$, 64%), 81 ([ImCH_2]$^+$, 43%).

High resolution mass : m/z 274.1253; calcd. for $C_{14}H_{18}N_4S$: 274.1252.

Anal. ($C_{14}H_{18}N_4S$•$C_2H_2O_4$) : 52.56 %C, 5.54 %H, 15.31 %N;
Calcd. : 52.73 %C, 5.53 %H, 15.37 %N.

N-*Methyl*-N'-*[3-(4(5)-imidazolyl)propyl]thiourea oxalate (VUF 4630) (**3.3a**)*

Melting point 126.1-128.9 °C. Yield 49%.

^{1}H NMR (D_2O) : δ 1.96 (m, 2H, $C\underline{H}_2CH_2NH$), 2.77 (t, J = 7 Hz, 2H, imidazole-$C\underline{H}_2$), 2.86 (br s, 3H, $C\underline{H}_3$), 3.30-3.67 (m, 2H, $C\underline{H}_2NH$), 7.23 (s, 1H, imidazole-5(4)H), 8.57 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 198 (M^+, 20%), 167 (M^+-CH_3NH_2, 6%), 164 (M^+-H_2S, 5%), 109 ([ImC_3H_6]$^+$, 12%), 95 ([ImC_2H_4]$^+$, 94%), 82 ([$ImCH_3$]$^+$, 100%).

High resolution mass : m/z 198.0929; calcd. for $C_8H_{14}N_4S$: 198.0939.

Anal. ($C_8H_{14}N_4S$•0.84$C_2H_2O_4$) : 42.43 %C, 5.41 %H, 20.75 %N;
Calcd. : 42.44 %C, 5.77 %H, 20.46 %N.

N-*Ethyl*-N'-*[3-(4(5)-imidazolyl)propyl]thiourea oxalate (VUF 4631) (**3.3b**)*

Melting point 116.1 °C. Yield 44%.

^{1}H NMR (D_2O) : δ 1.12 (t, 3H, J = 7 Hz, $C\underline{H}_3$), 1.95 (m, 2H, $C\underline{H}_2CH_2NH$), 2.77 (t, 2H, J = 8 Hz, imidazole-$C\underline{H}_2$), 3.15-3.62 (m, 4H, 2*$C\underline{H}_2NH$), 7.23 (s, 1H, imidazole-5(4)H), 8.57 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 212 (M^+, 53%), 178 (M^+-H_2S, 10%), 167 (M^+-$C_2H_5NH_2$, 4%), 109 ([ImC_3H_6]$^+$, 31%), 95 ([ImC_2H_4]$^+$, 85%), 82 ([$ImCH_3$]$^+$, 100%).

High resolution mass : m/z 212.1092; calcd. for $C_9H_{16}N_4S$: 212.1096.

Anal. ($C_9H_{16}N_4S$•$C_2H_2O_4$) : 43.93 %C, 5.91 %H, 18.74 %N;
Calcd. : 43.70 %C, 6.00 %H, 18.53 %N.

N-(n-*Propyl*)-N'-*[3-(4(5)-imidazolyl)propyl]thiourea oxalate (VUF 4632) (**3.3c**)*

Melting point 123.2-125.2 °C. Yield 24%.

^{1}H NMR (D_2O) : δ 0.87 (t, 3H, J = 7 Hz, $C\underline{H}_3$), 1.53 (m, 2H, $C\underline{H}_2CH_3$), 1.97 (m, 2H, $C\underline{H}_2CH_2NH$), 2.77 (t, 2H, J = 7 Hz, imidazole-$C\underline{H}_2$), 3.10-3.65 (m, 4H, 2*$C\underline{H}_2NH$), 7.23 (s, 1H, imidazole-5(4)H), 8.56 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 226 (M^+, 9%), 192 (M^+-H_2S, 4%), 167 (M^+-$C_3H_7NH_2$, 4%), 109 ([ImC_3H_6]$^+$, 9%), 95 ([ImC_2H_4]$^+$, 100%), 82 ([$ImCH_3$]$^+$, 33%).

High resolution mass : m/z 226.1265; calcd. for $C_{10}H_{18}N_4S$: 226.1252.

Anal. ($C_{10}H_{18}N_4S$•0.8$C_2H_2O_4$) : 46.95 %C, 6.31 %H, 18.79 %N;
Calcd. : 46.70 %C, 6.62 %H, 18.78 %N.

N-(iso-*Propyl*)-N'-*[3-(4(5)-imidazolyl)propyl]thiourea oxalate (VUF 4633)* ***(3.3d)***

Melting point 146.0 °C. Yield 43%.

^{1}H NMR (D_2O) : δ 1.13 (d, 6H, J = 7 Hz, C<u>H</u>$_3$), 1.94 (m, 2H, C<u>H</u>$_2$CH$_2$NH), 2.77 (t, 2H, J = 7 Hz, imidazole-C<u>H</u>$_2$), 3.37-3.58 (m, 2H, C<u>H</u>$_2$NH), 3.89-4.17 (m, 1H, C<u>H</u>), 7.23 (s, 1H, imidazole-5(4)H), 8.57 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 226 (M^+, 30%), 192 (M^+-H_2S, 6%), 167 (M^+-$C_3H_7NH_2$, 7%), 109 ([ImC_3H_6]$^+$, 23%), 95 ([ImC_2H_4]$^+$, 79%), 82 ([$ImCH_3$]$^+$, 66%).

High resolution mass : m/z 226.1271; calcd. for $C_{10}H_{18}N_4S$: 226.1252.

Anal. ($C_{10}H_{18}N_4S$•0.8$C_2H_2O_4$) : 46.52 %C, 6.55 %H, 19.15 %N;
Calcd. : 46.70 %C, 6.62 %H, 18.78 %N.

N-*Cyclohexyl*-N'-*[3-(4(5)-imidazolyl)propyl]thiourea oxalate (VUF 4634)* ***(3.3e)***

Melting point 102.2 °C. Yield 50%.

^{1}H NMR (D_2O) : δ 0.93-1.97 (m, 12H, C<u>H</u>$_2$CH$_2$NH + cyclohexyl-CH$_2$'s), 2.70 (t, 2H, J = 8 Hz, imidazole-C<u>H</u>$_2$), 3.23-3.90 (m, 3H, C<u>H</u>$_2$NH + C<u>H</u>NH), 7.18 (s, 1H, imidazole-5(4)H), 8.52 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 266 (M^+, 29%), 232 (M^+-H_2S, 8%), 167 (M^+-$C_6H_{11}NH_2$, 6%), 109 ([ImC_3H_6]$^+$, 24%), 95 ([ImC_2H_4]$^+$, 73%), 82 ([$ImCH_3$]$^+$, 100%).

High resolution mass : m/z 266.1572; calcd. for $C_{13}H_{22}N_4S$: 266.1565.

N-*Phenyl*-N'-*[3-(4(5)-imidazolyl)propyl]thiourea oxalate (VUF 4635)* ***(3.3f)***

Melting point 126.7 °C. Yield 47%.

^{1}H NMR (D_2O) : δ 1.90 (m, 2H, C<u>H</u>$_2$CH$_2$NH), 2.70 (t, 2H, J = 7 Hz, imidazole-C<u>H</u>$_2$), 3.39-3.65 (m, 2H, C<u>H</u>$_2$NH), 7.27 (m, 6H, imidazole-5(4)H + phenyl-H's), 8.50 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 260 (M^+, 2%), 226 (M^+-H_2S, 3%), 135 ([C_6H_5NCS]$^+$, 63%), 108 ([ImC_3H_5]$^+$, 19%), 95 ([ImC_2H_4]$^+$, 68%), 93 ([$C_6H_5NH_2$]$^+$, 74%), 82 ([$ImCH_3$]$^+$, 100%), 77 ([C_6H_5]$^+$, 30%).

High resolution mass : m/z 260.1108; calcd. for $C_{13}H_{16}N_4S$: 260.1096.

Anal. ($C_{13}H_{16}N_4S$•0.8$C_2H_2O_4$) : 52.59 %C, 5.26 %H, 16.94 %N;
Calcd. : 52.76 %C, 5.34 %H, 16.86 %N.

N-*Benzyl*-N'-*[3-(4(5)-imidazolyl)propyl]thiourea oxalate (VUF 4636) (3.3g)*

Melting point 117.2 °C. Yield 33%.

^{1}H NMR (D_2O) : δ 1.84 (m, 2H, $C\underline{H}_2CH_2NH$), 2.39-2.79 (m, 2H, imidazole-$C\underline{H}_2$), 3.30-3.57 (m, 2H, $C\underline{H}_2NH$), 4.42-4.73 (m, 2H, $C\underline{H}_2$-phenyl), 7.10 (s, 1H, imidazole-5(4)H), 7.29 (m, 5H, phenyl-H),8.47 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 274 (M^+, 50%), 240 (M^+-H_2S, 2%), 168 (M^+-C_7H_7NH, 10%), 109 ($[ImC_3H_6]^+$, 31%), 95 ($[ImC_2H_4]^+$, 79%), 91 ($[C_7H_7]^+$, 100%), 82 ($[ImCH_3]^+$, 94%).

High resolution mass : m/z 274.1250; calcd. for $C_{14}H_{18}N_4S$: 274.1252.

Anal. ($C_{14}H_{18}N_4S$•0.84$C_2H_2O_4$) : 53.69 %C, 5.59 %H, 16.39 %N;
Calcd. : 53.81 %C, 5.66 %H, 16.01 %N.

N-*(2-Phenylethyl)*-N'-*[3-(4(5)-imidazolyl)propyl]thiourea oxalate(VUF 4637) (3.3h)*

Melting point 125.5 °C. Yield 27%.

^{1}H NMR (D_2O) : δ 1.73 (m, 2H, $C\underline{H}_2CH_2NH$), 2.58 (t, 2H, J = 8 Hz, imidazole-$C\underline{H}_2$), 2.82 (t, 2H, J = 7 Hz, $C\underline{H}_2$-phenyl), 3.10-3.44 (m, 2H, $C\underline{H}_2NH$), 3.44-3.79 (m, 2H, $C\underline{H}_2CH_2$-phenyl), 7.12 (s, 1H, imidazole-5(4)H), 7.16-7.36 (m, 5H, phenyl-H), 8.49 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 288 (M^+, 0.2%), 95 ($[ImC_2H_4]^+$, 8%), 91 ($[C_7H_7]^+$, 47%), 82 ($[ImCH_3]^+$, 18%), 45 ($[C_2H_5NH_2]^+$, 100%).

High resolution mass : m/z 288.1414; calcd. for $C_{15}H_{20}N_4S$: 288.1409.

Anal. ($C_{15}H_{20}N_4S$•0.8$C_2H_2O_4$) : 55.34 %C, 5.83 %H, 15.79 %N;
Calcd. : 55.32 %C, 6.04 %H, 15.55 %N.

N-*Methyl*-N'-*[4-(4(5)-imidazolyl)butyl]thiourea oxalate (VUF 4680) (3.4a)*

Melting point 120.1-122.6 °C. Yield 18%.

^{1}H NMR (D_2O) : δ 1.61 (m, 4H, central CH_2's), 2.72 (t, 2H, J = 7 Hz, imidazole-$C\underline{H}_2$), 2.82 (m, 3H, $C\underline{H}_3$), 3.22-3.62 (m, 2H, $C\underline{H}_2NH$), 7.17 (s, 1H, imidazole-5(4)H), 8.52 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 212 (M^+, 79%), 181 (M^+-CH_3NH_2, 20%), 179 (M^+-HS, 9%), 123 ($[ImC_4H_8]^+$, 42%), 109 ($[ImC_3H_6]^+$, 43%), 95 ($[ImC_2H_4]^+$, 100%), 81 ($[ImCH_2]^+$, 69%).

High resolution mass : m/z 212.1091; calcd. for $C_9H_{16}N_4S$: 212.1096.

Anal. ($C_9H_{16}N_4S \cdot 0.8C_2H_2O_4$) : 44.74 %C, 6.33 %H, 19.34 %N;
Calcd. : 44.78 %C, 6.24 %H, 19.70 %N.

N-*Ethyl*-N'-*[4-(4(5)-imidazolyl)butyl]thiourea oxalate (VUF 4681) (3.4b)*

Melting point 120.3 °C. Yield 29%.

^{1}H NMR (D_2O) : δ 1.08 (t, 3H, J = 7Hz, C<u>H</u>$_3$), 1.61 (m, 4H, central CH_2's), 2.72 (t, 2H, J = 7 Hz, imidazole-C<u>H</u>$_2$), 3.22-3.51 (m, 4H, 2*C<u>H</u>$_2$NH), 7.17 (s, 1H, imidazole-5(4)H), 8.52 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 226 (M^+, 81%), 193 (M^+-HS, 7%), 181 (M^+-$C_2H_5NH_2$, 25%), 123 ([ImC_4H_8]$^+$, 47%), 109 ([ImC_3H_6]$^+$, 40%), 95 ([ImC_2H_4]$^+$, 100%), 81 ([$ImCH_2$]$^+$, 75%).

High resolution mass : m/z 226.1250; calcd. for $C_{10}H_{18}N_4S$: 226.1252.

Anal. ($C_{10}H_{18}N_4S \cdot 1.8C_2H_2O_4$) : 41.85 %C, 5.47 %H, 14.40 %N;
Calcd. : 42.06 %C, 5.60 %H, 14.42 %N.

N-(n-*Propyl*)-N'-*[4-(4(5)-imidazolyl)butyl]thiourea oxalate (VUF 4682) (3.4c)*

Melting point 146.9 °C. Yield 55%.

^{1}H NMR (D_2O) : δ 0.84 (t, 3H, J = 7Hz, C<u>H</u>$_3$), 1.42-1.78 (m, 6H, central CH_2's + C<u>H</u>$_2$$CH_3$), 2.73 (t, 2H, J = 7 Hz, imidazole-C<u>H</u>$_2$), 3.10-3.62 (m, 4H, 2*C<u>H</u>$_2$NH), 7.18 (s, 1H, imidazole-5(4)H), 8.53 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 240 (M^+, 69%), 207 (M^+-HS, 7%), 181 (M^+-$C_3H_7NH_2$, 23%), 123 ([ImC_4H_8]$^+$, 55%), 109 ([ImC_3H_6]$^+$, 38%), 95 ([ImC_2H_4]$^+$, 100%), 81 ([$ImCH_2$]$^+$, 80%), 45 ([$C_2H_5NH_2$]$^+$, 71%).

High resolution mass : m/z 240.1409; calcd. for $C_{11}H_{20}N_4S$: 240.1409.

Anal. ($C_{11}H_{20}N_4S \cdot C_2H_2O_4$) : 47.06 %C, 6.62 %H, 16.83 %N;
Calcd. : 47.25 %C, 6.71 %H, 16.96 %N.

N-(iso-*Propyl*)-N'-*[4-(4(5)-imidazolyl)butyl]thiourea oxalate (VUF 4683) (3.4d)*

Melting point 151.3 °C. Yield 64%.

^{1}H NMR (D_2O) : δ 1.16 (d, 6H, J = 7 Hz, 2*C$\underline{H}_3$), 1.65 (m, 4H, central CH_2's), 2.76 (t, 2H, J = 7 Hz, imidazole-C$\underline{H}_2$), 3.43 (m, 2H, C$\underline{H}_2$NH), 4.08 (m, 1H, C$\underline{H}$), 7.21 (s, 1H, imidazole-5(4)H), 8.55 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 240 (M^+, 60%), 207 (M^+-HS, 5%), 181 (M^+-$C_3H_7NH_2$, 17%), 123 ([ImC_4H_8]$^+$, 51%), 109 ([ImC_3H_6]$^+$, 25%), 95 ([ImC_2H_4]$^+$, 70%), 81 ([$ImCH_2$]$^+$, 52%), 45 ([$C_2H_5NH_2$]$^+$, 100%).

High resolution mass : m/z 240.1401; calcd. for $C_{11}H_{20}N_4S$: 240.1409.

Anal. ($C_{11}H_{20}N_4S$•1.76$C_2H_2O_4$) : 43.71 %C, 5.71 %H, 14.37 %N;
Calcd. : 43.73 %C, 5.94 %H, 14.05 %N.

N-*Cyclohexyl*-N'-*[4-(4(5)-imidazolyl)butyl]thiourea oxalate (VUF 4684) (3.4e)*

Melting point 109.5 °C. Yield 24%.

^{1}H NMR (DMSO-d_6) : δ 1.00-1.95 (m, 14H, central CH_2's + cyclohexyl-C$\underline{H}_2$'s), 2.64 (m, 2H, imidazole-C$\underline{H}_2$), 3.37 (m, 2H, C$\underline{H}_2$NH), 3.93 (m, 1H, C$\underline{H}$), 7.20 (s, 1H, imidazole-5(4)H), 7.28-7.62 (m, 4H, N$\underline{H}$ + $CO_2\underline{H}$), 8.50 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 280 (M^+, 66%), 247 (M^+-HS, 7%), 181 (M^+-$C_6H_{11}NH_2$, 28%), 123 ([ImC_4H_8]$^+$, 62%), 109 ([ImC_3H_6]$^+$, 32%), 95 ([ImC_2H_4]$^+$, 100%), 81 ([$ImCH_2$]$^+$, 76%), 45 ([$C_2H_5NH_2$]$^+$, 65%).

High resolution mass : m/z 280.1724; calcd. for $C_{14}H_{24}N_4S$: 280.1722.

Anal. ($C_{14}H_{24}N_4S$•1.15$C_2H_2O_4$) : 50.52 %C, 6.93 %H, 15.04 %N;
Calcd. : 50.98 %C, 6.90 %H, 14.60 %N.

N-*Phenyl*-N'-*[4-(4(5)-imidazolyl)butyl]thiourea oxalate (VUF 4685) (3.4f)*

Melting point 153.7 °C. Yield 42%.

^{1}H NMR (D_2O) : δ 1.59 (m, 4H, central CH_2's), 2.70 (t, 2H, imidazole-C$\underline{H}_2$), 3.49 (m, 2H, C$\underline{H}_2$NH), 7.31 (m, 6H, imidazole-5(4)H + phenyl-H), 8.50 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 274 (M^+, 4%), 241 (M^+-HS, 2%), 181 (M^+-$C_6H_5NH_2$, 10%), 135 ([C_6H_5NCS]$^+$, 100%), 95 ([ImC_2H_4]$^+$, 61%), 93 ([$C_6H_5NH_2$]$^+$, 78%), 77 ([C_6H_5]$^+$, 47%).

High resolution mass : m/z 274.1251; calcd. for $C_{14}H_{18}N_4S$: 274.1252.

Anal. ($C_{14}H_{18}N_4S \cdot 1.4C_2H_2O_4$) : 50.18 %C, 5.00 %H, 14.31 %N;
Calcd. : 50.39 %C, 5.24 %H, 14.00 %N.

N-*Benzyl*-N'-*[4-(4(5)-imidazolyl)butyl]thiourea oxalate (VUF 4686)* ***(3.4g)***

Melting point 109.1 °C. Yield 42%.

^{1}H NMR (DMSO-d_6) : δ 1.53 (m, 4H, central CH_2's), 2.59 (t, 2H, J = 7 Hz, imidazole-$C\underline{H}_2$), 3.40 (m, 2H, $C\underline{H}_2NH$), 4.63 (m, 2H, $C\underline{H}_2$-benzyl), 7.13 (s, 1H, imidazole-5(4)H), 7.28 (m, 5H, phenyl-H), 7.71 (m, 1H, N-H), 7.99 (m, 1H, N-H), 8.39 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 288 (M^+, 42%), 255 (M^+-HS, 3%), 181 (M^+-$C_7H_7NH_2$, 14%), 123 ($[ImC_4H_8]^+$, 28%), 109 ($[ImC_3H_6]^+$, 15%), 106 ($[C_7H_7NH]^+$, 38%), 95 ($[ImC_2H_4]^+$, 57%), 91 ($[C_7H_7]^+$, 100%), 81 ($[ImCH_2]^+$, 37%).

High resolution mass : m/z 288.1400; calcd. for $C_{15}H_{20}N_4S$: 288.1409.

Anal. ($C_{15}H_{20}N_4S \cdot 0.85C_2H_2O_4$) : 54.98 %C, 5.91 %H, 15.57 %N;
Calcd. : 54.96 %C, 5.99 %H, 15.35 %N.

N-*(2-Phenylethyl)*-N'-*[4-(4(5)-imidazolyl)butyl]thiourea oxalate (VUF 4687)* ***(3.4h)***

Melting point 130.8-132.2 °C. Yield 24%.

^{1}H NMR (DMSO-d_6) : δ 1.55 (m, 4H, central CH_2's), 2.62 (t, 2H, J = 7 Hz, imidazole-$C\underline{H}_2$), 2.78 (t, 2H, J = 7 Hz, $C\underline{H}_2$-phenyl), 3.37 (m, 2H, $C\underline{H}_2NH$), 3.57 (m, 2H, $C\underline{H}_2CH_2$-phenyl), 7.27 (m, 6H, imidazole-5(4)H + phenyl-H), 7.63 (m, 2H, 2*N-H), 8.67 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 302 (M^+, 55%), 269 (M^+-HS, 5%), 181 (M^+-$C_8H_9NH_2$, 26%), 123 ($[ImC_4H_8]^+$, 50%), 109 ($[ImC_3H_6]^+$, 32%), 105 ($[C_8H_9]^+$, 36%), 95 ($[ImC_2H_4]^+$, 91%), 91 ($[C_7H_7]^+$, 100%), 81 ($[ImCH_2]^+$, 66%).

High resolution mass : m/z 302.1560; calcd. for $C_{16}H_{22}N_4S$: 302.1565.

N-*Methyl*-N'-*[5-(4(5)-imidazolyl)pentyl]thiourea oxalate (VUF 4613)* ***(3.5a)***

Melting point 111.0 °C. Yield 10%.

^{1}H NMR (D_2O) : δ 1.31 (m, 2H, $(CH_2CH_2)_2C\underline{H}_2$), 1.62 (m, 4H, $(CH_2C\underline{H}_2)_2CH_2$), 2.67 (t, 2H, J = 7 Hz, imidazole-$C\underline{H}_2$), 2.82 (m, 3H, $C\underline{H}_3$), 3.33 (m, 2H, $C\underline{H}_2NH$), 7.14 (s, 1H, imidazole-5(4)H), 8.50 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 226 (M^+, 58%), 195 (M^+-CH_3NH_2, 58%), 137 ([ImC_5H_{10}]$^+$, 36%), 123 ([ImC_4H_8]$^+$, 25%), 109 ([ImC_3H_6]$^+$, 15%), 95 ([ImC_2H_4]$^+$, 93%), 82 ([$ImCH_3$]$^+$, 100%).

High resolution mass : m/z 226.1251; calcd. for $C_{10}H_{18}N_4S$: 226.1252.

N-*Ethyl*-N'-*[5-(4(5)-imidazolyl)pentyl]thiourea oxalate (VUF 4614)* ***(3.5b)***

Melting point 77.6 °C. Yield 8%.

^{1}H NMR (D_2O) : δ 1.12 (t, 3H, J = 7 Hz, $C\underline{H}_3$), 1.35 (m, 2H, $(CH_2CH_2)_2C\underline{H}_2$), 1.65 (m, 4H, $(CH_2C\underline{H}_2)_2CH_2$), 2.72 (t, 2H, J = 7 Hz, imidazole-$C\underline{H}_2$), 3.47 (m, 4H, 2*$C\underline{H}_2NH$), 7.19 (s, 1H, imidazole-5(4)H), 8.55 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 240 (M^+, 75%), 207 (M^+-HS, 5%), 195 (M^+-$C_2H_5NH_2$, 12%), 137 ([ImC_5H_{10}]$^+$, 52%), 123 ([ImC_4H_8]$^+$, 32%), 109 ([ImC_3H_6]$^+$, 20%), 95 ([ImC_2H_4]$^+$, 100%), 82 ([$ImCH_3$]$^+$, 95%).

High resolution mass : m/z 240.1410; calcd. for $C_{11}H_{20}N_4S$: 240.1409.

Anal. ($C_{11}H_{20}N_4S$•$0.8C_2H_2O_4$) : 48.55 %C, 6.95 %H, 17.74 %N;
Calcd. : 48.44 %C, 6.97 %H, 17.93 %N.

N-(n-*Propyl*)-N'-*[5-(4(5)-imidazolyl)pentyl]thiourea oxalate (VUF 4615)* ***(3.5c)***

Melting point 115.5-116.6 °C. Yield 7%.

^{1}H NMR (D_2O) : δ 0.82 (t, 3H, J = 7 Hz, $C\underline{H}_3$), 1.30 (m, 4H, $(CH_2CH_2)_2C\underline{H}_2$) + $C\underline{H}_2CH_3$), 1.60 (m, 4H, $(CH_2C\underline{H}_2)_2CH_2$), 2.67 (t, 2H, J = 7 Hz, imidazole-$C\underline{H}_2$), 3.30 (m, 4H, 2*$C\underline{H}_2NH$), 7.13 (s, 1H, imidazole-5(4)H), 8.50 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 254 (M^+, 60%), 221 (M^+-HS, 4%), 195 (M^+-$C_3H_7NH_2$, 12%), 137 ([ImC_5H_{10}]$^+$, 56%), 123 ([ImC_4H_8]$^+$, 28%), 109 ([ImC_3H_6]$^+$, 17%), 95 ([ImC_2H_4]$^+$, 100%), 82 ([$ImCH_3$]$^+$, 88%).

High resolution mass : m/z 254.1563; calcd. for $C_{12}H_{22}N_4S$: 254.1565.

Anal. ($C_{12}H_{22}N_4S$•$0.8C_2H_2O_4$) : 50.33 %C, 7.22 %H, 16.70 %N;
Calcd. : 50.04 %C, 7.28 %H, 17.16 %N.

N-(iso-*Propyl*)-N'-*[5-(4(5)-imidazolyl)pentyl]thiourea oxalate (VUF 4616)* ***(3.5d)***

Melting point 97.1 °C. Yield 8%.

^{1}H NMR (D_2O) : δ 1.08 (s, 6H, 2*C$\underline{H}_3$), 1.28 (m, 2H, $(CH_2CH_2)_2C\underline{H}_2$), 1.57 (m, 4H, $(CH_2C\underline{H}_2)_2CH_2$), 2.65 (t, 2H, imidazole-C$\underline{H}_2$), 3.32 (m, 2H, C$\underline{H}_2$NH), 4.00 (m, 1H, C$\underline{H}$), 7.13 (s, 1H, imidazole-5(4)H), 8.47 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 254 (M^+, 77%), 196 (M^+-C_3H_7NH, 18%), 137 ($[ImC_5H_{10}]^+$, 74%), 123 ($[ImC_4H_8]^+$, 27%), 95 ($[ImC_2H_4]^+$, 100%), 81 ($[ImCH_2]^+$, 80%).

High resolution mass : m/z 254.1563; calcd. for $C_{12}H_{22}N_4S$: 254.1565.

Anal. ($C_{12}H_{22}N_4S$•0.8$C_2H_2O_4$) : 50.09 %C, 7.06 %H, 17.07 %N;
Calcd. : 50.04 %C, 7.28 %H, 17.16 %N.

N-*Cyclohexyl*-N'-*[5-(4(5)-imidazolyl)pentyl]thiourea oxalate (VUF 4617)* ***(3.5e)***

Melting point 116.5 °C. Yield 6%.

^{1}H NMR (D_2O) : δ 1.05-2.05 (m, 16H, $(CH_2CH_2)_2C\underline{H}_2$ + $(CH_2C\underline{H}_2)_2CH_2$ + cyclohexyl-C$\underline{H}_2$'s), 2.73 (t, 2H, J = 7 Hz, imidazole-C$\underline{H}_2$), 3.43 (m, 3H, C$\underline{H}_2$NH + C$\underline{H}$NH), 7.22 (s, 1H, imidazole-5(4)H), 8.58 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 294 (M^+, 45%), 261 (M^+-HS, 4%), 195 (M^+-$C_6H_{11}NH_2$, 16%), 137 ($[ImC_5H_{10}]^+$, 48%), 123 ($[ImC_4H_8]^+$, 22%), 109 ($[ImC_3H_6]^+$, 17%), 95 ($[ImC_2H_4]^+$, 100%), 82 ($[ImCH_3]^+$, 85%).

High resolution mass : m/z 294.1875; calcd. for $C_{15}H_{26}N_4S$: 294.1878.

Anal. ($C_{15}H_{26}N_4S$•0.8$C_2H_2O_4$) : 54.52 %C, 7.48 %H, 15.34 %N;
Calcd. : 54.40 %C, 7.59 %H, 15.29 %N.

N-*Phenyl*-N'-*[5-(4(5)-imidazolyl)pentyl]thiourea oxalate (VUF 4618)* ***(3.5f)***

Melting point 108.9 °C. Yield 5%.

^{1}H NMR (D_2O) : δ 1.35 (m, 2H, $(CH_2CH_2)_2C\underline{H}_2$), 1.67 (m, 4H, $(CH_2C\underline{H}_2)_2CH_2$), 2.75 (t, 2H, J = 7 Hz, imidazole-C$\underline{H}_2$), 3.53 (m, 2H, C$\underline{H}_2$NH), 7.37 (m, 6H, imidazole-5(4)H + phenyl-H), 8.59 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 288 (M^+, 5%), 196 (M^+-C_6H_5NH, 14%), 137 ($[ImC_5H_{10}]^+$, 18%), 135 ($[C_6H_5NCS]^+$, 100%), 123 ($[ImC_4H_8]^+$, 7%), 109 ($[ImC_3H_6]^+$, 13%), 95 ($[ImC_2H_4]^+$, 84%), 93 ($[C_6H_5NH_2]^+$, 37%), 82 ($[ImCH_3]^+$, %), 77 ($[C_6H_5]^+$, 52%).

High resolution mass : m/z 288.1402; calcd. for $C_{15}H_{20}N_4S$: 288.1409.

Anal. ($C_{15}H_{20}N_4S$•$C_2H_2O_4$) : 54.18 %C, 6.09 %H, 14.91 %N;
Calcd. : 53.95 %C, 5.86 %H, 14.80 %N.

N-*Benzyl*-N'-*[5-(4(5)-imidazolyl)pentyl]thiourea oxalate (VUF 4619)* ***(3.5g)***

Melting point 152.1-152.4 °C. Yield 10%.

^{1}H NMR (D_2O) : δ 1.27 (m, 2H, $(CH_2CH_2)_2C\underline{H}_2$), 1.60 (m, 4H, $(CH_2C\underline{H}_2)_2CH_2$), 2.67 (t, 2H, J = 7 Hz, imidazole-$C\underline{H}_2$), 3.43 (m, 2H, $C\underline{H}_2NH$), 4.63 (m, 2H, $C\underline{H}_2$-phenyl), 7.17 (s, 1H, imidazole-5(4)H), 7.36 (m, 5H, phenyl-H), 8.53 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 302 (M^+, 38%), 269 (M^+-HS, 3%), 195 (M^+-$C_7H_7NH_2$, 11%), 137 ($[ImC_5H_{10}]^+$, 30%), 123 ($[ImC_4H_8]^+$, 14%), 109 ($[ImC_3H_6]^+$, 11%), 106 ($[C_7H_7NH]^+$, 32%), 95 ($[ImC_2H_4]^+$, 74%), 91 ($[C_7H_7]^+$, 100%), 81 ($[ImCH_2]^+$, 56%).

High resolution mass : m/z 302.1560; calcd. for $C_{16}H_{22}N_4S$: 302.1565.

Anal. ($C_{16}H_{22}N_4S$•$C_2H_2O_4$)	: 54.89 %C, 6.12 %H, 14.20 %N;
Calcd.	: 55.09 %C, 6.16 %H, 14.27 %N.

N-*(2-Phenylethyl)*-N'-*[5-(4(5)-imidazolyl)pentyl]thiourea oxalate (VUF 4620)* ***(3.5h)***

Melting point 118.5-119.5 °C. Yield 5%.

^{1}H NMR (D_2O) : δ 1.14-1.78 (m, 6H, $(CH_2(C\underline{H}_2)_3CH_2)$, 2.72 (t, 2H, J = 7 Hz, imidazole-$C\underline{H}_2$), 2.88 (t, 2H, J = 7 Hz, $C\underline{H}_2$-phenyl), 3.32 (m, 2H, $C\underline{H}_2NH$), 3.67 (m, 2H, $C\underline{H}_2CH_2$-phenyl), 7.17 (s, 1H, imidazole-5(4)H), 7.32 (m, 5H, phenyl-H), 8.52 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 316 (M^+, 33%), 283 (M^+-HS, 3%), 196 (M^+-C_8H_9NH, 22%), 137 ($[ImC_5H_{10}]^+$, 50%), 123 ($[ImC_4H_8]^+$, 23%), 109 ($[ImC_3H_6]^+$, 15%), 105 ($[C_8H_9]^+$, 41%), 95 ($[ImC_2H_4]^+$, 61%), 91 ($[C_7H_7]^+$, 94%), 82 ($[ImCH_3]^+$, 100%).

High resolution mass : m/z 316.1716; calcd. for $C_{17}H_{24}N_4S$: 316.1722.

Anal. ($C_{17}H_{24}N_4S$•$C_2H_2O_4$)	: 56.32 %C, 6.55 %H, 13.94 %N;
Calcd.	: 56.14 %C, 6.45 %H, 13.78 %N.

N-*(4-Chloro-benzyl)*-N'-*[5-(4(5)-imidazolyl)pentyl]thiourea oxalate (VUF 4742)* ***(3.5i)***

Melting point 139.1-139.9 °C. Yield 43%.

^{1}H NMR (DMSO-d_6) : δ 1.29 (m, 2H, $(CH_2CH_2)_2C\underline{H}_2$), 1.56 (m, 4H, $(CH_2C\underline{H}_2)_2CH_2$), 2.68 (t, 2H, J = 8 Hz, imidazole-$C\underline{H}_2$), 3.37 (m, 2H, $C\underline{H}_2NH$), 4.63 (m, 2H, $C\underline{H}_2$-phenyl), 7.17 (s, 1H, imidazole-5(4)H), 7.29 (d, 2H, 2,6-phenyl-H), 7.49 (d, 2H, 3,5-phenyl-H), 7.68 (m, 1H, $N\underline{H}$-CH_2), 7.98 (m, 1H, $N\underline{H}$-benzyl), 8.53 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 336 (M^+-2, 0.07%), 127 ($[C_7H_6Cl^{37}]^+$, 32%), 125 ($[C_7H_6Cl^{35}]^+$, 100%).

High resolution mass : m/z 336.1177; calcd. for $C_{16}H_{21}N_4SCl$: 336.1176.

Anal. ($C_{16}H_{21}N_4SCl \bullet C_2H_2O_4$)	: 50.70 %C, 5.48 %H, 13.19 %N;
Calcd.	: 50.64 %C, 5.43 %H, 13.12 %N.

N-*Methyl*-N'-*[6-(4(5)-imidazolyl)hexyl]thiourea oxalate (VUF 4740)* ***(3.6a)***

Melting point 106.5-110.0 °C. Yield 18%.

^{1}H NMR (D_2O) : δ 1.20-1.78 (m, 8H, $CH_2(C\underline{H}_2)_4CH_2$), 2.72 (t, 2H, J = 7Hz, imidazole-$C\underline{H}_2$), 2.90 (s, 3H, $C\underline{H}_3$-N), 3.18-3.58 (m, 2H, $C\underline{H}_2NH$), 7.18 (s, 1H, imidazole-4(5)H), 8.53 (s, 1H, imidazole- 2H) ppm.

MS (EI) : m/z 240 (M^+, 44%), 210 (M^+-CH_4N, 7%), 151 ($[ImC_6H_{12}]^+$, 56%), 137 ($[ImC_5H_{10}]^+$, 38%), 123 ($[ImC_4H_8]^+$, 12%), 109 ($[ImC_3H_6]^+$, 19%), 95 ($[ImC_2H_4]^+$, 100%), 82 ($[ImCH_3]^+$, 78%), 81 ($[Im\text{-}CH_2]^+$, 78%), 45 ($[C_2H_7N]^+$, 45%).

High resolution mass : m/z 240.1411; calcd. for $C_{11}H_{20}N_4S$: 240.1409.

Anal. ($C_{11}H_{20}N_4S \bullet C_2H_2O_4$)	: 46.98 %C, 6.66 %H, 16.87 %N;
Calcd.	: 47.26 %C, 6.71 %H, 16.96 %N.

N-*Phenyl*-N'-*[6-(4(5)-imidazolyl)hexyl]thiourea oxalate (VUF 4741)* ***(3.6f)***

Melting point 126.7-130.2 °C. Yield 8%.

^{1}H NMR (D_2O) : δ 1.22-1.79 (m, 8H, im-$CH_2(C\underline{H}_2)_4CH_2$), 2.70 (t, 2H, J = 7 Hz, imidazole-$C\underline{H}_2$), 3.34-3.59 (m, 2H, $C\underline{H}_2NH$), 7.09-7.52 (m, 6H, phenyl-H + imidazole-4(5)H), 8.50 (s, 1H, imidazole-2H) ppm.

MS (EI) : m/z 302 (M^+, 1%), 151 ($[ImC_6H_{12}]^+$, 36%), 137 ($[ImC_5H_{10}]^+$, 15%), 123 ($[ImC_4H_8]^+$, 7%), 109 ($[ImC_3H_6]^+$, 16%), 95 ($[ImC_2H_4]^+$, 100%), 82 ($[ImCH_3]^+$, 84%), 81 ($[Im\text{-}CH_2]^+$, 72%), 77 ($[C_6H_5]^+$, 2%), 45 ($[C_2H_7N]^+$, 1%).

High resolution mass : m/z 302.1562; calcd. for $C_{16}H_{22}N_4S$: 302.1565.

Anal. ($C_{16}H_{22}N_4S \cdot C_2H_2O_4$) : 55.01 %C, 6.00 %H, 14.42 %N;
Calcd. : 55.09 %C, 6.16 %H, 14.28 %N.

Pharmacology

The histamine H_3 activity was determined on an *in vitro* assay, using a guinea pig jejunum preparation, according to literature procedures.[15] Each compound was tested on tissue preparations of at least four different animals in triplicate. The potency of the antagonists was expressed by its pA_2 value, calculated from the Schild regression analysis, and at least three different concentrations were used. Statistical analysis was carried out with the Students' *t*-test and $p < 0.05$ was considered statistically significant. The Schild slopes were not significantly different from unity.

The affinity of the described compounds for the H_1 receptor was determined in at least three independent experiments, performed in triplicate, by the displacement of [^{3}H]mepyramine, bound to membranes of CHO cells expressing guinea pig H_1 receptors.[122]

The affinity of the described compounds for the H_2 receptor was established in at least three independent experiments, performed in triplicate, by the displacement of [^{125}I]iodoaminopotentidine, bound to membranes of CHO cells expressing human H_2 receptors.[123]

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Chapter 9

The Effects of Analogues of Immepip and Thioperamide on the Histamine H_3 Receptor

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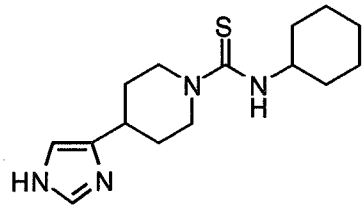
Introduction

The H_2 agonist impromidine and the H_2 antagonist burimamide were among the first, antagonists with reasonable potency, found for the histamine H_3 receptor; lack of selectivity made them less attractive as pharmacological tools for this receptor.[5] Not long after the discovery of the histamine H_3 receptor, its existence was confirmed by the development of the H_3 selective agonist (R)-α-methylhistamine and the H_3 selective antagonist thioperamide (**1**).[6]

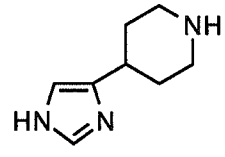


1, thioperamide

The potent and selective H_3 antagonist thioperamide (**1**) was developed from a series of rigid analogues of histamine. This compound comprises several distinct structural features, also present in the structure of burimamide, such as an alkyl substituted thiourea group linked through an alkyl chain to the 4(5)-position of an imidazole ring. The cyclohexyl group in the structure of thioperamide (**1**) appears to be favoured for a high H_3 affinity (see Chapter 2; Table 20).[48]

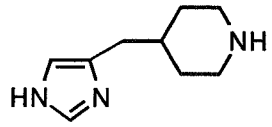
Further modifications on the structure of thioperamide (**1**), like substitutions on the imidazole ring, only resulted in loss of activity,[58,60] although a methyl group on the 5-position of the imidazole only impairs a small decrease of the antagonistic activity (pA_2 value is 8.4, compared to 9.0 for thioperamide (guinea pig ileum); see Chapter 2; Table 21).[58]

The substituted thiourea function of thioperamide appears to be very important for a high affinity, because replacement of this moiety by an amino group leads to the weak antagonist **2**, with a pA_2 value of 5.7 (rat cortex; see Chapter 2; Table 15).[26]



2

Recently, we reported another rigid analogue of histamine; immepip (**3a**). This compound also contains a piperidine ring, just as in the structure of, thioperamide (**1**) and **2**. The only difference in structure, compared to the weak H_3 antagonist **2** is the introduction of a methylene spacer between the imidazole ring and the piperidine ring. Yet immepip is a potent and selective histamine H_3 agonist, with a pD_2 value of 8.0 (guinea pig jejunum; see Chapter 6).[42]



3a, immepip

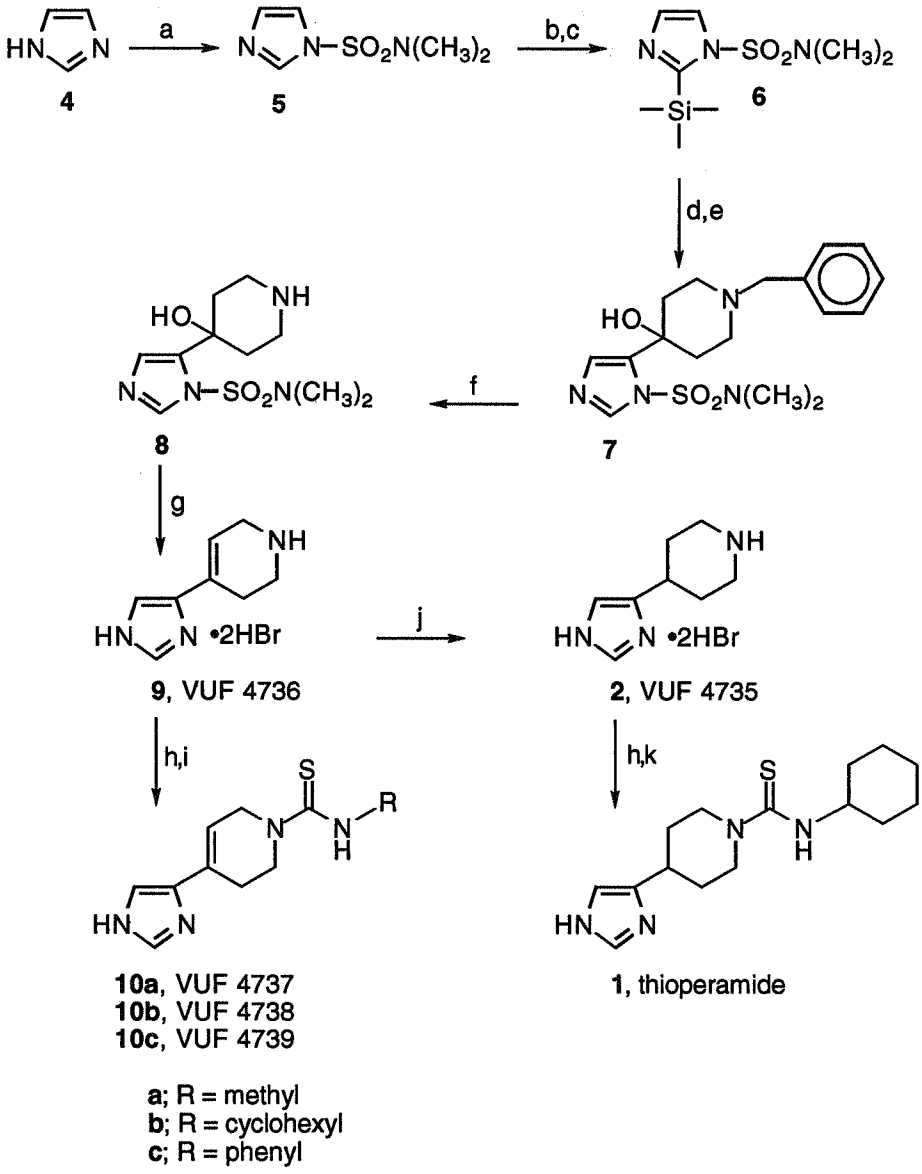
Apparently a basic amino function is important for activation of the receptor, but the position of the amino group relative to the imidazole ring is very critical.

In order to obtain more information about the optimal location and orientation of the amino group, relative to the imidazole ring, we have prepared a series of analogues of immepip (**3a**). In this series the position of the amino group on the piperidine ring of immepip (**3a**) has been altered (compound **3b** and **3c**).
Moreover the piperidine ring was replaced by the smaller pyrrolidine ring. leading to compounds **20** and **23**. The amino group in these compounds is separated from the imidazole ring by a butylene spacer, as in immepip (**3a**), or by a propylene chain, as in **2**, depending on the spatial orientation of the group.
In addition we prepared a few, more rigid analogues of thioperamide (**1**), by the replacement of the piperidine ring with a 1,2,3,6-tetrahydropyridine ring.

For the synthesis of the compounds with a six-membered ring, a synthesis route was developed, based on the direct coupling of a suitable electrophile to a 1,2-diprotected imidazole. In addition, this route proved to be an elegant, inexpensive new method for the synthesis of thioperamide, on a preparative scale.
The pyrrolidine derivatives were prepared by the addition of a metallated 1-protected-2-pyrrolidinone to a 1-protected-imidazol-4-ylcarboxaldehyde.

Chemistry

Thioperamide analogues



Scheme 1. Reagents used: (a) *N,N*-Dimethylsulfamoyl chloride, Et_3N, toluene; (b) *n*-BuLi, THF, -70°C; (c) trimethylsilyl chloride; (d) *n*-BuLi, THF, -70°C; (e) 1-benzyl-4-piperidone; (f) Pd/C, HCO_2NH_4, methanol, reflux; (g) 30% HBr, reflux; (h) Na, ethanol, reflux; (i) R-NCS; (j) Pd/C, 20 atm H_2, methanol; (k) cyclohexylisothiocyanate.

Thioperamide (**1**) and a few, more rigid analogues (**10a-c**) were prepared by the addition of 1-benzyl-4-piperidone to lithiated 1,2-diprotected imidazole **6** (see scheme 1). The *N,N*-dimethylsulfamoyl group was used for the protection of the 1-position of the imidazole ring (see Chapter 5). For the protection of the 2-position the trimethylsilyl group was used. The *tert*-butyldimethylsilyl group can be used effectively as well, but is more expensive. The trimethylsilyl group is hydrolyzed during the aqueous workup.

The benzyl group of the resulting alcohol **7** was effectively removed by a catalytic transfer hydrogenation, using ammonium formate as hydrogen donor with palladium as catalyst.[132,133]

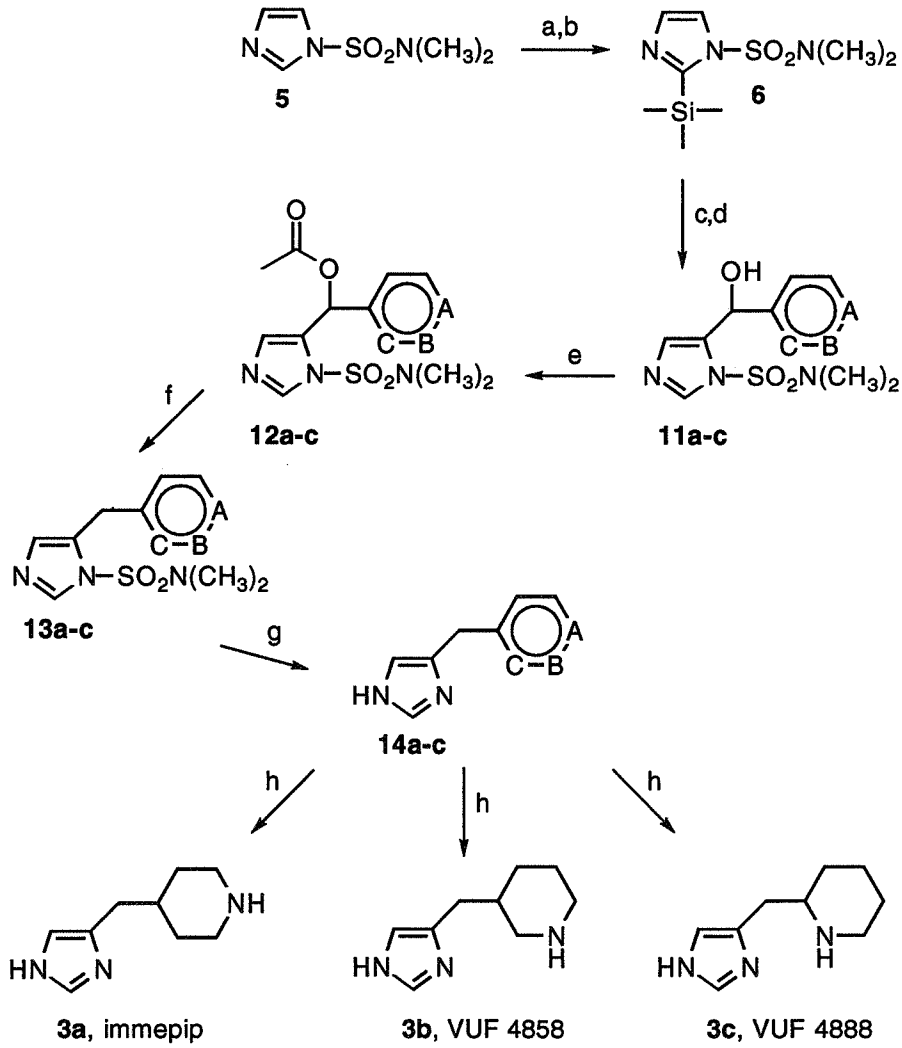
Dehydration and deprotection of **8** to the 1,2,3,6-tetrahydropyridine derivative **9** (VUF 4736) was easily achieved by acidic hydrolysis.

The rigid thioperamide analogues **10a-c** were prepared, starting from this compound **9**, by the addition of the corresponding isothiocyanates (see Chapter 8).

Reduction of the double bond of VUF 4736 (**9**) by hydrogenation with palladium as catalyst, led to VUF 4735 (**2**) (overall yield, 50%).

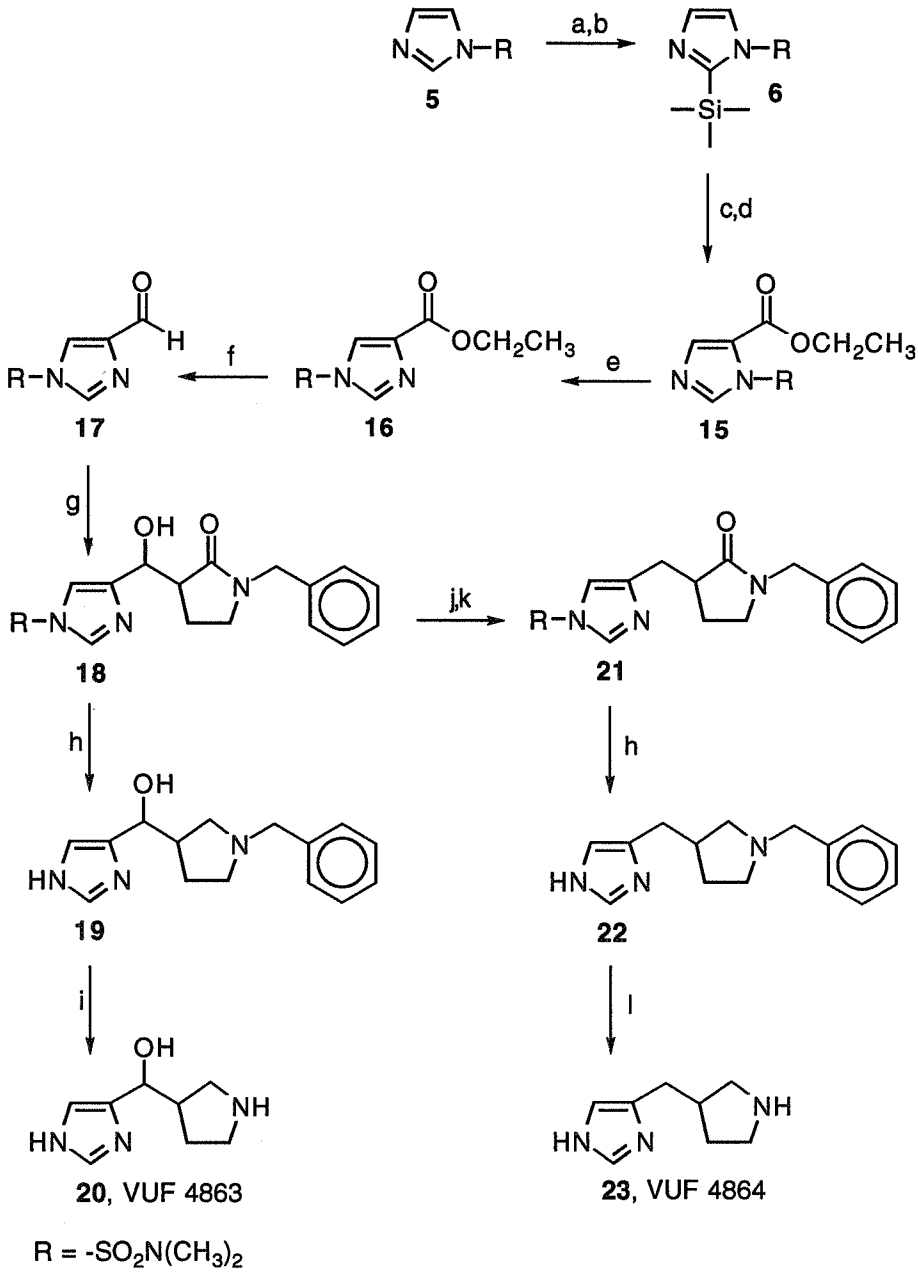
Thioperamide (**1**) can be prepared from the reaction of VUF 4735 (**2**) with cyclohexylisothiocyanate.

Immepip analogues



Scheme 2. Reagents used: (a) *n*-BuLi, THF, -70°C; (b) trimethylsilyl chloride; (c) *n*-BuLi, THF, -70°C; (d) pyridinecarboxaldehyde; (e) Et_3N, DMAP, acetic anhydride, DCM; (f) Et_3N, Pd/C, 50 atm H_2, EtOH; (g) 30% HBr, reflux; (h) Pd/C, 50 atm H_2, 50% EtOH.

The analogues of immepip (**3b,c**) were prepared as shown in scheme 2. This route is based on the method described for the synthesis of immepip (**3a**) (see Chapter 6).[42] The lithiated 1,2-diprotected imidazole **6** was treated with the corresponding pyridinecarboxaldehyde. The trimethylsilyl group hydrolyzed during workup.
The alcohol **11** was treated with acetic anhydride under basic conditions and the acetate group of the resulting compound **12** was removed by hydrogenation. Hydrolysis of this compound under acidic conditions led to the deprotected derivative **14**, which was subsequently hydrogenated, using palladium as catalyst, to **3a-c**.
The main difference with the method described for the synthesis of immepip (**3a**), as described in Chapter 6 is the selective removal of the acetate group by hydrogenolysis under basic conditions. For the synthesis of immepip (**3a**) as described in Chapter 6, this was achieved by hydrogenolysis under acidic conditions, which resulted in the reduction of the pyridine ring as well. The selective hydrogenolysis under basic conditions made it possible to isolate the unprotected pyridine derivatives **14a-c**.



Scheme 3. Reagents used: (a) *n*-BuLi, THF, -70°C; (b) trimethylsilyl chloride; (c) *n*-BuLi, THF, -70°C; (d) $ClCO_2Et$; (e) acidic workup; (f) DIBAL-H, toluene, -60°C; (g) 1-benzyl-3-lithio-2-pyrrolidinone, THF, -60°C; (h) $LiAlH_4$, THF, reflux; (i) Pd/C, HCO_2NH_4, methanol, reflux; (j) $POCl_3$, pyridine, reflux; (k) Pd/C, 15 atm H_2, EtOH; (l) $Pd(OH)_2$, 35 atm H_2, MeOH.

The key step in the synthesis of the pyrrolidine derivatives **20** and **23** was the successful coupling of 1-benzyl-3-lithio-pyrrolidinone with 1-protected-imidazol-4-ylcarboxaldehyde **17**.

A few methods have been described in literature for the synthesis of imidazole derivatives with a carboxaldehyde function on the 4- or 5 position, starting from imidazole.[93,100,103,104,134] In these procedures a lithiated imidazole derivative, prepared either by halogen-metal exchange[103,104,134] or by direct lithiation,[93,100] was quenched with DMF.*

Our attempts to quench a 1,2-diprotected 5-lithiated imidazole **6** with DMF were successful on a small scale (1-10 mmol). However, low yields and difficult workup procedures were encountered during large scale reactions, which prompted us to search for a preparative alternative.

Quenching of **6** with chloro ethylformate and subsequent workup led to compound **16** with the ethyl carboxylate function on the 4-position. This thermodynamically more stable 4-ester derivative **16** was found to be the result of a rearrangement of **15** under the acidic workup conditions. Under neutral to basic workup conditions, the 5-ester derivative **15** was isolated. The 4-ester derivative **16** was used for further reactions, because the ester function could be readily reduced to the aldehyde using diisobutylaluminum hydride (DIBAL-H) at low temperatures. The reduction of the isomeric 5-ester derivative proved to be difficult (low yield and more side products) probably due to steric hindrance of the *N,N*-dimethylsulfamoyl group.

The addition of aldehyde **17** to 1-benzyl-3-lithio-2-pyrrolidinone resulted in alcohol **18**, from which both the pyrrolidine derivatives **20** and **23** were prepared.

The reduction of the amide of **18** by $LiAlH_4$ and subsequent basic workup, led to the removal of the *N,N*-dimethylsulfamoyl group as well. Reduction of the amide function using BH_3•THF or $NaBH_4$ in CF_3CO_2H failed.

The hydroxymethyl pyrrolidine derivative of immepip **20**, was obtained by debenzylation of **19** by a catalytic transfer hydrogenation, using ammonium formate as hydrogen donor and palladium as catalyst.[132,133]

The hydroxyl group of **18** was removed by dehydration with $POCl_3$ in pyridine followed by subsequent hydrogenation of the alkene intermediate, to give compound **21**. The amide function of **21** was reduced successfully, using $LiAlH_4$ as described above. Debenzylation of **22** by catalytic transfer hydrogenation was unsuccessful. Hydrogenation with $Pd(OH)_2$ as catalyst, under 35 atm H_2, resulted in a clean formation of pyrrolidine analogue **23**.

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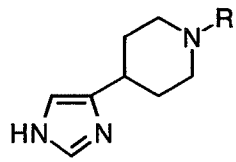
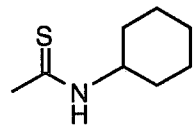
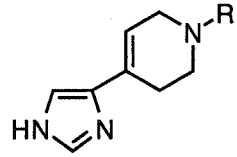
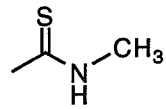
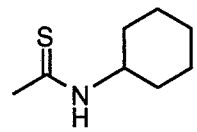
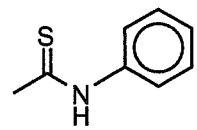
* The reported formation by Winter *et al.*[100] of a 1,2-diprotected-4-carboxaldehyde imidazole derivative from a 1,2-diprotected-5-lithio imidazole by quenching with DMF, seems to be based on a wrong structure assignment. If indeed the 4-carboxaldehyde derivative was isolated, a rearrangement must have taken place, perhaps during work-up.

Table 1. H_3 activity of the described analogues of thioperamide, as determined on guinea pig jejunum.

No.	Compound	Structure	R	pA_2 value	Schild -slope	N[a]
2	VUF 4735		H	6.2	0.8	4
1	thioper-amide			8.9	1.0	4
9	VUF 4736		H	5.4	0.9	4
10a	VUF 4737			5.4	0.7	3
10b	VUF 4738			6.3	0.9	5
10c	VUF 4739			5.6	0.9	4

[a] Number of different animals used.

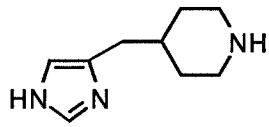
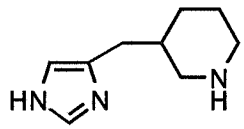
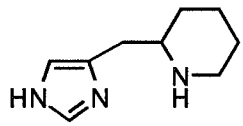
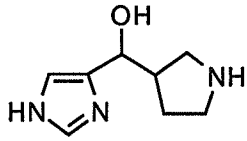
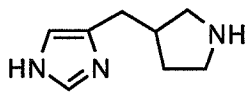
Pharmacology

The H_3 activity of the compounds was determined on an *in vitro* test system, on the basis of the concentration dependent inhibitory effect of histamine H_3 agonists on the electrically evoked contractile response of isolated guinea pig jejunum segments (see Chapter 3).[15]

Results and Discussion

All the compounds have been tested for H_3 agonism and H_3 antagonism.
The analogues of thioperamide (**1**) all displayed full reversible antagonism on the histamine H_3 receptor, as determined on guinea pig jejunum (see table 1).
Further reduction of the conformational flexibility of VUF 4735 (**2**) by the introduction of a double bond, is clearly not favourable. The piperidine derivative VUF 4735 (**2**) is almost ten times more potent as an H_3 antagonist than its more rigid 1,2,3,6-tetrahydropyridine analogue VUF 4736 (**9**).
The extraordinary increase of antagonistic activity when the amino group of VUF 4735 (**2**) is replaced by a *N*-cyclohexyl thiourea moiety, leading to thioperamide (**1**), is not observed with the tetrahydropyridine analogues. This must be the result of the reduced conformational flexibility as well; apparently the thiourea function can not assume the same orientation, relatively to the imidazole ring as in thioperamide. Nevertheless, although not as extreme as going from VUF 4735 (**2**) to thioperamide, an increase of affinity is observed going from VUF 4736 to the *N*-cyclohexyl thiourea derivative VUF 4738 (**10b**). This compound is almost ten times more potent than its precursor. Furthermore, VUF 4738 (**10b**) is more potent than its methyl- or phenyl analogue. This favourable behaviour of the cyclohexyl group was observed for thioperamide (**1**) as well (see Chapter 2). Apparently this part of the ligand binds to a hydrophobic site of the receptor; a π-π interaction is not likely.

Table 2. H_3 activity of the described analogues of immepip, as determined on guinea pig jejunum.

No.	Compound	Structure	H_3 activity	N[a]
3a	immepip		$pD_2 = 8.0 \pm 0.1$	8
3b	VUF 4858[b]		$pA_2 = 6.5 \pm 0.2$[c]	2
3c	VUF 4888[b]		$pA_2 < 5.0$[c]	2
20	VUF 4863[b]		$pD_2 = 5.5 \pm 0.2$ ($\alpha = 0.6 \pm 0.2$)	2
23	VUF 4864[b]		$pD_2 = 7.3 \pm 0.1$ ($\alpha = 0.8 \pm 0.1$)	2

[a] Number of different animals used.
[b] Unresolved mixture of enantiomers.
[c] No agonistic activity was observed.

The H_3 activities of the analogues of immepip (**3a**) were determined on the guinea pig jejunum and are represented in table 2.

Replacement of the 4-piperidine ring of immepip by a 3-piperidine ring is clearly disastrous for H_3 activity, since VUF 4858 (**3b**) does not display any agonistic activity and is a weak H_3 antagonist. This suggests that the amino group of VUF 4858 (**3b**) can not be positioned as optimal as the amino group of immepip (**3a**), relatively to the imidazole ring, verifying the strict structural requirements for an H_3 agonist. This information can be very useful for further SAR and molecular modelling studies, since certain low energy conformations of several potent H_3 agonist (e.g. histamine derivatives and immepip) matching those of VUF 4858, can be excluded now.

The 2-piperidine derivative of immepip, VUF 4888 (**3c**), which can be regarded as a rigid analogue of histamine, has a negligible affinity for the H_3 receptor. This is less surprising, because substitution of the amino group of histamine is only allowed with small alkyl groups, e.g. N^{α}-propylhistamine has also been described to be inactive (see chapter 2; table 6). This verifies the difference between immepip and e.g. histamine as an H_3 agonist and indicates that immepip can not be regarded as an analogue of histamine.

The replacement of the 4-piperidine ring of immepip (**3a**) by a 3-pyrrolidine ring however is allowed and VUF 4864 (**23**) proved to be a rather good H_3 agonist with a pD_2 value of 7.3 and an intrinsic activity of about 0.8. This compound has also been reported in a patent application,[31] to displace [^{3}H] N^{α}-methylhistamine on guinea pig brain tissue, with a pK_i value of 7.0 (see Chapter 2; table 11). VUF 4864 can be fitted on the potent H_3 agonist immepip (**3a**), as well as on the weak H_3 antagonist VUF 4858 (**3b**). Apparently VUF 4864 (**23**) can obtain a comparable conformation as immepip (**3a**) and activate the H_3 receptor. For this reason, also VUF 4864 (**23**) is very useful as a tool for SAR and molecular modelling studies, but has to be resolved first.

Experimental Section

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC-200 (200 MHz) spectrometer with tetramethylsilane or sodium 3-(trimethylsilyl)-propionate as an internal standard. Mass spectra were recorded on a Finnigan MAT-90. Melting points were measured on a Mettler FP-5 + FP-52 apparatus and are uncorrected. THF was distilled from $LiAlH_4$. *n*-Butyllithium was purchased from Janssen Chimica as a 15% solution in hexane.

1-(*N,N*-Dimethylsulfamoyl)imidazole **5** was synthesized by the method described by Chadwick and Ngochindo,[90] with the exception that toluene was used as a solvent instead of benzene.

The synthesis of immepip (**3a**) is described in Chapter 6.

*1-Benzyl-4-[1-(*N,N-*dimethylsulfamoyl)imidazol-5-yl]-4-hydroxypiperidine* (**7**)

10.0 g (57 mmol) 1-(*N,N*-Dimethylsulfamoyl)imidazole (**5**) was dissolved in 200 ml dry THF under an atmosphere of dry nitrogen and cooled to -70°C. *n*-Butyllithium in hexane (35 ml, 57 mmol) was added dropwise (temperature should not exceed -65°C). After 15 min, a solution of trimethylsilyl chloride (6.2 g, 57 mmol) in 50 ml dry THF was added and the solution was stirred at room temperature for 1 h. The mixture was cooled to -70°C again and *n*-butyllithium in hexane (35 ml, 57 mmol) was added dropwise (temperature should not exceed -65°C). After 1 h, a solution of *N*-benzyl-4-piperidone (10.8 g, 5.7 mmol) in dry THF was added gradually and the mixture was allowed to (slowly) warm to room temperature overnight. The reaction mixture was poured into 500 ml water and the THF was removed under reduced pressure. The product was extracted with dichloromethane (3 x 250 ml), dried on Na_2SO_4 and concentrated *in vacuo*. The residue (25.0 g) was purified by column chromatography with a mixture of ethyl acetate and petroleum ether (60/80) (ratio = 1/1) as eluent. 18.0 g (87%) Light-yellow crystals (R_f = 0.1) where isolated.

^{1}H NMR ($CDCl_3$) : δ 2.72-3.51 (m, 8H, Pip-H), 2.97(s, 6H, NCH_3), 4.37 (s, 1H, OH), 4.42 (s, 2H, CH_2), 7.28 (s, 1H, Im-4H), 7.38-7.60 (m, 5H, Phen-H), 8.68 (s, 1H, Im-2H) ppm.

*4-[1-(*N,N-*Dimethylsulfamoyl)imidazol-5-yl]-4-hydroxypiperidine* (**8**)

1.0 g (2.7 mmol) **7** was dissolved in 25 ml methanol. 0.1 gram 10% Pd/C and 1.0 g (15.9 mmol) ammoniumformate was added. The mixture was refluxed for 1 h. After filtration over a short celite column, the solution was concentrated *in vacuo* and 0.7 g (93%) of a white solid was isolated.

^{1}H NMR ($CDCl_3$) : δ 1.80-1.98 (m, 2H, Pip-3,5-H_{ax}), 2.03-2.19 (m, 2H, Pip-3,5-H_{eq}), 2.81-2.88 (m, 2H, Pip-2,6-H_{ax}), 2.97 (s, 6H, NCH_3), 2.94-3.16 (m, 3H, Pip-2,6-H_{eq} + OH), 6.92 (s, 1H, Im-4H), 7.70 (s, 1H, Im-2H) ppm.

4-(Imidazol-4(5)-yl)-1,2,3,6-tetrahydropyridine dihydrobromide (VUF 4736) (**9**)

2.5 g (9 mmol) **8** was dissolved in 30% HBr (50 ml) and refluxed for 16 h. The HBr-solution was removed under pressure and the residue was refluxed for 2 h in 50 ml absolute ethanol. A white precipitate was collected by centrifugation and dried. The supernatant was concentrated *in vacuo* and the residue was washed with acetone (3 x 50 ml). The remaining light beige solid and the white precipitate were combined

and recrystallized from methanol/ethanol. 2.1 g (74%; 60% overall) White crystals where collected. Melting point : 271-275 °C.

^{1}H NMR (D_2O) : δ 2.77 (m, 2H, THP-3H), 3.52 (t, 2H, J 6.7 Hz, THP-2H), 3.90 (s, 2H, THP-6H), 6.36 (m, 1H, THP-5H), 7.52 (s, 1H, Im-4(5)H), 8.74 (s, 1H, Im-2H) ppm.

^{13}C NMR (D_2O) : δ 23.5, 41.8, 43.2, 116.9, 119.8, 124.6 , 133.2, 135.7 ppm.

MS (EI) : m/e 149 (M^+, 100%), 148 (M^+-H, 68%), 120 (M^+-CH_3N, 24%), 119 (M^+-CH_4N, 43%), 94 ($[C_5H_6N_2]^+$, 22%), 80 ($[C_5H_6N]^+$, 48%), 67 ($[C_3H_3N_2]^+$, 10%).

N-*Methyl*-N'-*[4-(imidazol-4(5)-yl)-1,2,3,6-tetrahydropyridyl]thiourea oxalate (VUF 4737)* (**10a**)

0.7 g (2.2 mmol) VUF 4736 (**9**), was added to 2 equivalent sodium ethanolate in 50 ml absolute ethanol. This solution was refluxed for 1 h and cooled to room temperature. The formed precipitate was removed by filtration and 1.0 g (13.7 mmol) methylisothiocyanate was added to the filtrate. The ethanol was removed under reduced pressure, after stirring overnight. The residue was purified by column-chromatography by washing with a mixture of ethyl acetate and petroleum ether (60/80) (ratio = 1/1) (isothiocyanate eluted R_f = 1.0). The product was subsequently eluted with methanol as eluent. The methanol was removed under reduced pressure and the free base was dissolved in ethyl acetate/*iso*-propanol. A saturated oxalic acid solution in ethyl acetate was added dropwise to the free base solution, under vigorous stirring, until pH<3. A white precipitate formed, which was collected by centrifugation, washed with ethyl acetate (three times) and recrystallized from absolute ethanol/ethyl acetate. Yield 0.7 g (83%). Melting point 148-151 °C.

^{1}H NMR (D_2O) : δ 2.58 (m, 2H, THP-3H), 3.01 (s, 3H, CH_3), 4.00 (t, 2H, J 6.7 Hz, THP-2H), 4.29 (s, 2H, THP-6H), 6.27 (s, 1H, THP-5H), 7.42 (s, 1H, Im-4(5)H), 8.61 (s, 1H, Im-2H) ppm.

^{13}C NMR (D_2O) : δ 26.7, 33.7, 45.7, 48.2, 116.0, 123.4, 125.0, 134.1, 135.3, 181.0 ppm.

N-*Cyclohexyl*-N'-*[4-(imidazol-4(5)-yl)-1,2,3,6-tetrahydropyridyl]thiourea oxalate (VUF 4738)* (**10b**)

The same procedure and amounts were used as described for the synthesis of VUF 4737 (**10a**), but this time 1.0 g (7.0 mmol) cyclohexylisothiocyanate was added. Yield 0.6 g (70%). Melting point 121-123.5°C.

^{1}H NMR (D_2O) : δ 1.01-1.40 (m, 6H, cyclohexyl-3,4,5-H), 1.51-1.94 (m, 5H, cyclohexyl-1,2,6-H), 2.51 (m, 2H, THP-3H), 4.03 (t, 2H, J 6.7 Hz, THP-2H), 4.37 (s, 2H, THP-6H), 6.25 (s, 1H, THP-5H), 7.43 (s, 1H, Im-4(5)H), 8.61 (s, 1H, Im-2H) ppm.

N-*Phenyl*-N'-*[4-(imidazol-4(5)-yl)-1,2,3,6-tetrahydropyridyl]thiourea oxalate (VUF 4739)* (**10c**)

The same procedure and amounts were used as described for the synthesis of VUF 4737 (**10a**), but this time 1.0 g (7.4 mmol) phenylisothiocyanate was added. Yield 0.7 g (83%). Melting point 115-118°C.

^{1}H NMR (D_2O) : δ 2.60 (m, 2H, THP-3H), 4.14 (t, 2H, J 6.7 Hz, THP-2H), 4.51 (s, 2H, THP-6H), 6.32 (s, 1H, THP-5H), 7.13-7.48 (m, 6H, Phen-H + Im-4(5)H), 8.66 (s, 1H, Im-2H) ppm.

4-(Imidazol-4(5)-yl)piperidine dihydrobromide (VUF 4735) (**2**)

1.0 g (3.2 mmol) VUF 4736 (**9**) was dissolved in 75 ml methanol. 0.1 g 10% Pd/C was added and stirred for 16 h under 20 atm hydrogen pressure in an autoclave. The reaction mixture was filtrated over a short celite column, concentrated *in vacuo* and washed with absolute ethanol. 1.0 g (100%; 60% overall) White crystals where isolated. Melting point : 275-278 °C.

^{1}H NMR (D_2O) : δ 2,03 (m, 2H, Pip-3,5-H_{ax}), 2.39 (dm, 2H, J 13 Hz, Pip-3,5-H_{eq}), 3.29 (m, 3H, Pip-2,6-H_{ax} + Pip-4H_{ax}), 3.63 (dm, 2H, J 13 Hz, Pip-2,6-H_{eq}), 7.48 (s, 1H, Im-4(5)H), 8.77 (s, 1H, Im-2H) ppm.

^{13}C NMR (D_2O) : δ 28.8, 31.3, 45.0, 116.4, 135.0, 137.0 ppm.

MS (EI) : m/z 151 (M^+, 20%), 123 (M^+-CH_2N, 9%), 95 (M^+-$C_2H_4N_2$, 100%), 82 ($[C_5H_8N]^+$, 23%), 67 ($[C_3H_3N_2]^+$, 5%), 28 ($[CH_2N]^+$, 10%).

N-*Cyclohexyl*-N'-*[4-(imidazol-4(5)-yl)-piperidyl]thiourea maleate (thioperamide)* (**1**)

The same procedure and amounts were used as described for the synthesis of VUF 4737 (**10a**), but this time 3 equiv cyclohexylisothiocyanate was added to VUF 4735 (**2**). Instead of oxalic acid, maleic acid in 2-propanol was added. Yield 60%. Melting point 115.6 °C.

^{1}H NMR (DMSO-d_6) : δ 1.01-2.00 (m, 14H, cyclohex-CH_2 + Pip-3,5-H), 2.88-3.13 (m, 3H, Pip-2,6-H_{ax} + Pip-4H_{ax}), 4.19 (bs, 1H, cyclohex-CH), 4.73 (dm, 2H, J 13 Hz, Pip-2,6-H_{eq}), 6.06 (s, maleic acid), 7.28 (d, 1H, J 7.7 Hz, NH), 7.42 (s, 1H, Im-4(5)H), 8.38 (s, 1H, Im-2H) ppm.

*3-[1-(*N,N-*Dimethylsulfamoyl)imidazol-5-ylhydroxymethyl]pyridine* (**11b**)

25.4 g (145 mmol) 1-(*N,N*-Dimethylsulfamoyl)imidazole (**5**) was dissolved in 400 ml dry THF under an atmosphere of dry nitrogen and cooled to -70°C. *n*-Butyllithium in hexane (100 ml, 0.16 mmol) was added dropwise (temperature should not exceed -65°C). Trimethylsilyl chloride (20.3 ml, 0.16 mol) was added after 15 min and the solution was subsequently stirred at room temperature for 1 h. The mixture was cooled to -70°C again and *n*-butyllithium in hexane (95 ml, 0.15 mol) was added dropwise (temperature should not exceed -65°C). After 1 h, 15.0 ml 3-pyridinecarboxaldehyde (0.16 mol) was added gradually and the mixture was allowed to (slowly) warm to room temperature overnight.
The solution was poured into 100 ml water and the THF was removed under reduced pressure. 400 ml 1M HCl was added and the water layer was extracted with ether (3 x 150 ml). After neutralization of the solution by addition of K_2CO_3, the product was extracted with ethyl acetate (5 x 100 ml). The ethyl acetate layers were combined. dried on Na_2SO_4 and concentrated *in vacuo*. The residue (37.0 g) was purified by recrystallization from ethyl acetate, resulting in 22.0 g (54%) white crystals.

^{1}H NMR ($CDCl_3$) : δ 2.89 (s, 6H, NCH_3), 4.90 (br s, 1H, OH), 6.10 (s, 1H, CH), 6.53 (s, 1H, Im-4H), 7.25 (dd, 1H, Pyr-5H), 7.76 (dt, 1H, Pyr-4H), 7.86 (s, 1H, Im-2H), 8.47 (dd, 1H, Pyr-6H), 8.53 (d, 1H, Pyr-2H) ppm.

^{13}C NMR ($CDCl_3$) : δ 38.0, 64.5, 123.4, 130.6, 134.4, 134.5, 136.4, 139.1, 147.9, 149.0 ppm.

*3-[1-(*N,N-*Dimethylsulfamoyl)imidazol-5-ylacetoxymethyl]pyridine* (**12b**)

18.8 g (67 mmol) Of the alcohol **11b** was dissolved in a solution of 0.85 g (7 mmol) 4-dimethylaminopyridine (DMAP) and 20.0 ml (144 mmol) triethylamine in 400 ml dichloromethane. After cooling the solution on an ice-bath, 9.5 ml (100 mmol) acetic anhydride was added dropwise and the reaction mixture was stirred for 90 min at room temperature. The mixture was subsequently washed with H_2O (3 x 100 ml), dried on Na_2CO_3 and concentrated *in vacuo*, resulting in 21.6 g (100%) of a yellow oil, which was used further without purification.

^{1}H NMR ($CDCl_3$) : δ 2.06 (s, 3H, CH_3CO), 2.87 (s, 6H, NCH_3), 6.90 (s, 1H, Im-4H), 7.05 (s, 1H, CH), 7.23 (dd, 1H, Pyr-5H), 7.63 (dt, 1H, Pyr-4H), 7.86 (s, 1H, Im-2H), 8.54 (dd, 1H, Pyr-6H), 8.60 (d, 1H, Pyr-2H) ppm.

^{13}C NMR ($CDCl_3$) : δ 20.7, 37.9, 66.8, 123.3, 130.1, 131.1, 133.0, 135.0, 139.0, 148.8, 149.8, 169.3 ppm.

*3-[1-(*N,N-*Dimethylsulfamoyl)imidazol-5-ylmethyl]pyridine* (**13b**)

26.9 g Of the acetate **12b** was dissolved in 100 ml ethanol and 10 ml triethylamine. 2.6 g 5% Pd/C Was added and this mixture was stirred under hydrogen pressure (50 atm) for 24 h in an autoclave. The catalyst was removed by filtration over a short celite column and the solvents were removed under reduced pressure. The pale brown residing oil was dissolved in 200 ml dichloromethane, washed with 100 ml 1M K_2CO_3-solution, dried on Na_2SO_4 and concentrated *in vacuo*. The resulting light brown oil (20.5 g, 94%) was purified by column chromatography using a mixture of ethyl acetate and methanol (ratio 9/1) as eluent. Yield 16.82 g (76%) of a pale yellow oil (R_f (MeOH) = 0.55).

^{1}H NMR ($CDCl_3$) : δ 2.72 (s, 6H, NCH_3), 4.04 (s, 2H, CH_2), 6.57 (s, 1H, Im-4H), 7.20 (dd, 1H, Pyr-5H), 7.48 (dt, 1H, Pyr-4H), 7.85 (s, 1H, Im-2H), 8.40-8.50 (m, 2H, Pyr-2,6-H) ppm.

^{13}C NMR ($CDCl_3$) : δ 28.0, 37.5, 123.2, 129.9, 130.0, 132.7, 136.1, 138.3, 147.9, 149.6 ppm.

3-(Imidazol-5-ylmethyl)pyridine (**14b**)

16.3 g (61 mmol) Of **13b** was refluxed in 150 ml 30% HBr for 24 h. After removal of the solvent under reduced pressure, the residue was dissolved in 100 ml absolute ethanol. This solution was refluxed for 3 h, concentrated *in vacuo* and the product was recrystallized from ethanol. Yield 16.2 g (82%) of a grey solid.

^{1}H NMR (D_2O) : δ 4.43 (s, 2H, CH_2), 7.34 (s, 1H, Im-4(5)H), 8.03 (dd, 1H, Pyr-5H), 8.51 (dt, 1H, Pyr-4H), 8.67 (s, 1H, Im-2H), 8.70-8.80 (m, 2H, Pyr-2,6-H) ppm.

^{13}C NMR (D_2O) : δ 28.5, 118.9, 129.0, 130.9, 135.6, 138.9, 141.7, 142.4, 148.8 ppm.

3-(Imidazol-4(5)-ylmethyl)piperidine (VUF 4858) (**3b**)

1.0 g 10% Pd/C was added to a solution of 10.0 g (31 mmol) **14b** in 50% ethanol and this suspension was stirred for 48 h under hydrogen-pressure (50 atm) in an autoclave. After filtration over a short celite column, the solution was concentrated *in vacuo*. The crude product was recrystallized from methanol, resulting in 9.23 g (90%) of a white solid.

^{1}H NMR (D_2O) : δ 1.15-1.40 (m, 1H, Pip-5H_{ax}), 1.50-2.00 (m, 3H, Pip-5H_{eq} + Pip-4H), 2.00-2.25 (m ,1H, Pip-3H), 2.65-3.00 (m, 4H, Pip-2,6-H_{ax} + Im-CH_2), 3.30-3.55 (m, 2H, Pip-2,6-H_{eq}), 7.28 (s, 1H, Im-4(5)H), 8.57 (s, 1H, Im-2H) ppm.

^{13}C NMR (D_2O) : δ 23.1, 28.9, 29.2, 34.6, 45.6, 49.3, 118.1, 131.6, 134.7 ppm.

*2-[1-(*N,N*-Dimethylsulfamoyl)imidazol-5-ylhydroxymethyl]pyridine* (**11c**)

The same procedure was used as described for the synthesis of **11b**, starting with 1.75 g (10 mmol) 1-(*N,N*-dimethylsulfamoyl)imidazole (**5**) and 0.95 ml (10 mmol) 2-pyridinecarboxaldehyde. The residue after hydrolysis was purified by recrystallization from ethyl acetate. Yield 1.3 g (46%) of white crystals.

^{1}H NMR ($CDCl_3$) : δ 3.00 (s, 6H, NCH_3), 5.15 (d, 1H, OH), 6.10 (s, 1H, CH), 6.45 (s, 1H, Im-4H), 7.20-7.40 (m, 2H, Pyr-3H + Pyr-5H), 7.72 (dt, 1H, Pyr-4H), 7.90 (s, 1H, Im-2H), 8.60 (m, 1H, Pyr-6H) ppm.

*2-[1-(*N,N-*Dimethylsulfamoyl)imidazol-5-ylacetoxymethyl]pyridine* (**12c**)

The same procedure was used as described for the synthesis of **12b**. Yield 100% of a yellow oil, which was used further without purification.

^{1}H NMR ($CDCl_3$) : δ 2.15 (s, 3H, CH_3CO), 295 (s, 6H, NCH_3), 6.80 (s, 1H, Im-4H), 7.10 (s, 1H, CH), 7.20-7.40 (m, 2H, Pyr-3H + Pyr-5H), 7.72 (dt, 1H, Pyr-4H), 7.95 (s, 1H, Im-2H), 8.60 (m, 1H, Pyr-6H) ppm.

*2-[1-(*N,N-*Dimethylsulfamoyl)imidazol-5-ylmethyl]pyridine* (**13c**)

The same procedure was used as described for the synthesis of **13b**. Yield 68%.

^{1}H NMR ($CDCl_3$) : δ 2.82 (s, 6H, NCH_3), 4.30 (s, 2H, CH_2), 6.68 (s, 1H, Im-4H), 7.05-7.30 (m, 2H, Pyr-3H + Pyr-5H), 7.60 (dt, 1H, Pyr-4H), 7.95 (s, 1H, Im-2H), 8.55 (m, 1H, Pyr-6H) ppm.

2-(Imidazol-5-ylmethyl)pyridine (**14c**)

The same procedure was used as described for the synthesis of **14b**. Yield 50%.

^{1}H NMR (D_2O) : δ 4.51 (s, 2H, CH_2), 7.45 (s, 1H, Im-4(5)H), 7.85-8.00 (m, 2H, Pyr-3H + Pyr-5H), 8.55 (dt, 1H, Pyr-4H), 8.67 (s, 1H, Im-2H), 8.71 (m, 1H, Pyr-6H) ppm.

2-(Imidazol-4(5)-ylmethyl)piperidine (VUF 4888) (**3c**)

The same procedure was used as described for the synthesis of **3b**. Yield 75%.

^{1}H NMR (D_2O) : δ 1.40-2.05 (dm, 6H, Pip-3H + Pip-4H + Pip 5H), 2.90-3.20 (m ,3H, CH_2 + Pip-6H_{ax}), 3.30-3.55 (m, 2H, Pip-2H + Pip-6H_{eq} + Im-CH_2), 7.40 (s, 1H, Im-4(5)H), 8.65 (s, 1H, Im-2H) ppm.

^{13}C NMR (D_2O) : δ 22.8, 23.1, 29.3, 29.8, 46.6, 57.0, 119.4, 128.5, 135.5 ppm.

*Ethyl [1-(*N,N-*dimethylsulfamoyl)imidazol-4-yl]carboxylate* (**16**)

27.0 g (0.15 mol) 1-(*N,N*-Dimethylsulfamoyl)imidazole **5** was dissolved in 250 ml dry THF under an atmosphere of dry nitrogen and cooled to -70°C. *n*-Butyllithium in hexane (100 ml, 0.16 mol) was added dropwise (temperature should not exceed -65°C). After 15 min, a solution of trimethylsilyl chloride (17.1 g, 0.16 mol) in 20 ml dry THF was added and the solution was stirred at room temperature for 2 h. The mixture was cooled to -70°C again and *n*-butyllithium in hexane (100 ml, 0.16 mol) was added dropwise (temperature should not exceed -65°C). After 1 h, 16.3 g ethyl chloroformate (0.15 mol) was added and the mixture was allowed to (slowly) warm to room temperature. The reaction mixture was poured into 250 ml water and the THF was removed under reduced pressure. The product was extracted with dichloromethane (3 x 150 ml), dried on Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in 200 ml ether and stirred for 1 h with 200 ml 0.5 M HCl. The ether layer was separated, dried on Na_2SO_4 and concentrated *in vacuo*. 34.5 g (91%) Of a light oil remained.

^{1}H NMR ($CDCl_3$) : δ 1.29 (t, 3H, J 6.7 Hz, CH_3), 2.79 (s, 6H, NCH_3), 4.29 (q, 2H, J 6.7 Hz, CH_2), 7.79 (s, 1H, Im-5H), 7.81 (s, 1H, Im-2H) ppm.

^{13}C NMR ($CDCl_3$) : δ 14.1, 37.9, 60.9, 122.6, 134.7, 136.7, 161.5 ppm.

MS (EI) : 247 (M^+, 17%), 202 (M^+-OCH_2CH_3, 28%), 175 (M^+-$CO_2CH_2CH_3$, 44%), 139 (M^+-SO_2NMe_2, 4%), 108 ($[SO_2NMe_2]^+$, 100%), 66 ($[Im]^+$, 1%), 44 ($[N(CH_3)_2]^+$, 11%).

*Ethyl [1-(*N,N-*dimethylsulfamoyl)imidazol-5-yl]carboxylate* (**15**)

15 was prepared employing the same procedure and amounts as for the synthesis of **16**. However the workup procedure was different. This time the reaction mixture was poured into 250 ml 5% $NaHCO_3$ solution and this was stirred overnight. The organic layer was separated and the water layer was additionally extracted with ether (3 x 75 ml). The combined organic layers were dried on Na_2SO_4 and concentrated *in vacuo*. The product was purified by column chromatography, using a mixture of ethyl acetate and petroleum ether (60/80) (ratio 1:1) (R_f = 0.2). Yield 30.0 g (78%).

^{1}H NMR ($CDCl_3$) : δ 1.32 (t, 3H, J 7.3 Hz, CH_3), 3.00 (s, 6H, NCH_3), 4.28 (q, 2H, J 6.7 Hz, CH_2), 7.69 (s, 1H, Im-5H), 8.09 (s, 1H, Im-2H) ppm.

^{13}C NMR ($CDCl_3$) : δ 13.9, 38.3, 60.9, 123.3, 139.0, 143.8, 157.8 ppm.

MS (EI) : 247 (M^+, 14%), 202 (M^+-OCH_2CH_3, 24%), 175 (M^+-$CO_2CH_2CH_3$, 40%), 139 (M^+-SO_2NMe_2, 4%), 108 ($[SO_2NMe_2]^+$, 100%), 66 ($[Im]^+$, 3%), 44 ($[N(CH_3)_2]^+$, 12%).

*[1-(*N,N*-Dimethylsulfamoyl)imidazol-4-yl]carboxaldehyde* (**17**)

16.0 g (65 mmol) **16** Was dissolved in 250 ml dry THF (150 ml) under an atmosphere of dry nitrogen and cooled to -60°C. 64.0 ml (78 mmol) diisobutylaluminum hydride in toluene (DIBAL-H) was added dropwise. The solution was poured in 200 ml 1 M HCl-solution, after 2 h stirring at -60°C. The solution was treated with K_2CO_3 to pH ~ 11 after 0.5 h. The organic layer was separated, dried on Na_2SO_4 and concentrated *in vacuo*. Recrystallization from *iso*-propanol resulted in 10.1 g (77%) white crystals. Melting point 105.4-107.0 °C.

^1H NMR ($CDCl_3$) : δ 2.86 (s, 6H, NCH_3), 7.84 (s, 1H, Im-5H), 7.91 (s, 1H, Im-2H), 9.88 (s, 1H, CH) ppm.

^{13}C NMR ($CDCl_3$) : δ 38.02, 122.24, 137.38, 141.99, 185.43 ppm.

MS (EI) : 203 (M^+, 23%), 108 ($[SO_2NMe_2]^+$, 100%), 66 ($[Im]^+$, 3%), 44 ($[N(CH_3)_2]^+$, 23%).

*1-Benzyl-3-[1-(*N,N*-dimethylsulfamoyl)imidazol-4-ylhydroxymethyl]-2-pyrrolidinone* (**18**)

1-Benzyl-2-pyrrolidinone (13.9 g, 87 mmol) was dissolved in dry THF (400 ml) under an atmosphere of dry nitrogen, and cooled to -65°C. n-Butyllithium (87 mmol)was added dropwise (temperature should not exceed -60°C). The solution was stirred at -60°C for 1 h and 11.8 g (58 mmol) (1-(*N,N*-dimethylsulfamoyl)imidazol-4-yl)carboxaldehyde (**17**) in THF (60 ml) was added dropwise. After 1 h the cooling bath was removed and the reaction mixture was allowed to warm to room temperature. 200 ml 1 M HCl was added and the mixture was stirred for 1 h. The solution was treated with K_2CO_3 to pH ~ 11 and the organic layer was separated. The water layer was extracted with dichloromethane (3 x 80 ml). The organic layers were combined, dried on Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column-chromatography using ethyl acetate (removal of 1-benzyl-2-pyrrolidinone; R_f = 0.2), followed by methanol as eluent. The methanol was removed under reduced pressure resulting in 16.9 g (77%) of a white solid. Melting point 145.2-148.0 °C.

^{1}H NMR ($CDCl_3$) : δ 1.70-2.08 (m, 2H, Pyrr-4H), 2.73 (s, 6H, NCH_3), 3.02-3.17 (m, 3H, Pyrr-3H + Pyrr-5H), 4.35 (dd, 2H, J 10.0 Hz, CH_2), 4.80 (d, 1H, J 7.3 Hz, CH), 5.26 (d, 1H, J 4.7 Hz, OH), 7.06-7.29 (m, 6H, Phen-H + Im-5H), 7.78 (s, 1H, Im-2H) ppm.

^{13}C NMR ($CDCl_3$) : δ 17.97, 37.98, 45.08, 46.46, 47.22, 66.87, 114.51, 127.31-128.59, 135.85, 145.57, 174.92 ppm.

1-Benzyl-3-(imidazol-4(5)-ylhydroxymethyl)pyrrolidine (**19**)

$LiAlH_4$ (0.20 g, 5.3 mmol) was added in small portions to a solution of **18** (0.5 g, 1.3 mmol) in THF (60 ml) at 0°C under a dry nitrogen atmosphere. The mixture was refluxed for 48 h and was subsequently cooled to 0°C again. 0.2 ml H_2O and 0.2 ml 4 M NaOH were added. The white/grey precipitate was removed by filtration and the filtrate was dried and concentrated *in vacuo*. Yield 0.3 g (88%).

^{1}H NMR ($CDCl_3$) : δ 1.42-1.93 (m, 2H, Pyrr-4H), 2.11-2.88 (m, 1H, Pyrr-3H), 2.11-2.88 (m, 4H, Pyrr-2H + Pyrr-5H), 3.56 (s, 2H, CH_2), 4.68 (d, 1H, J 2.7 Hz, CH), 6.72 (s, 1H, Im-5H), 7.01-7.61 (m, 5H, Phen-H)7.01-7.61 (m, 1H, Im-2H) ppm.

3-(Imidazol-4(5)-ylhydroxymethyl)pyrrolidine dioxalate (VUF 4863) (**20**)

0.1 g 10% Pd/C and 0.7 g (11.1 mmol) ammonium formate were added to a solution of 0.4 g (1.4 mmol) **19** in 20 ml methanol. The mixture was refluxed for 1 h and subsequently filtrated through a short celite column. The methanol was removed under reduced pressure and the residue (0.2 g, 100%) was dissolved in *iso*-propanol. This solution was added dropwise to a saturated oxalic acid solution in *iso*-propanol. The precipitate was collected by centrifugation and recrystallized from methanol/ethyl acetate. Melting point 173.1-176.9 °C.

^{1}H NMR (D_2O) : δ 1.92-2.29 (dm, 2H, Pyrr-4H), 2.82-3.02 (m, 1H, Pyrr-3H), 3.02-3.61 (m, 4H, Pyrr-2H + Pyrr-5H), 4.86-5.06 (m, 1H, CH), 7.46 (s, 1H, Im-5H), 8.69 (s, 1H, Im-2H) ppm.

*1-Benzyl-3-[1-(*N,N-*dimethylsulfamoyl)imidazol-4-ylmethyl]-2-pyrrolidinone* (**21**)

0.7 ml (7.3 mmol) $POCl_3$ was added slowly to a cooled (0°C) solution of 2.8 g (7.3 mmol) **18** in 60 ml dry pyridine. After stirring at room temperature overnight, the mixture was additionally refluxed for 4h. 60 ml H_2O was added and the product was extracted with ether (5x50 ml). The organic layers were dried on Na_2SO_4 and concentrated *in vacuo*. 1.2 g (46%) Of a light yellow oil was obtained.

^1H NMR ($CDCl_3$) : δ 2.82 (s, 6H, NCH_3), 3.01-3.14 (m, 2H, Pyrr-4H), 3.32 (t, 2H, J 6.0 Hz, Pyrr-5H), 4.55 (s, 2H, CH_2), 7.03-7.33 (m, 7H, Phen-H + =CH + Im-5H), 7.83 (s, 1H, Im-2H) ppm.

^{13}C NMR ($CDCl_3$) : δ 24.24, 38.05, 44.04, 47.15, 60.23, 117.93, 119.75, 127.49, 128.09, 128.55, 132.24, 136.80, 140.35, 168.66 ppm.

0.41 g (1.1 mmol) of this light oil was dissolved in 30 ml absolute ethanol and 0.3 g 5% Pd/C was added. This was stirred for 72 h under hydrogen pressure (15 atm) in an autoclave. The mixture was filtrated over a short celite column and the ethanol was removed under reduced pressure. Yield 0.26 g (63%).

^1H NMR ($CDCl_3$) : δ 1.62-2.25 (dm, 2H, Pyrr-4H), 2.82 (s, 6H, NCH_3), 2.94-3.23 (m, 2H, Im-CH_2), 2.94-3.23 (m, 3H, Pyrr-3H + Pyrr-5H), 4.40 (q, 2H, J 14.7 Hz, CH_2), 7.05-7.35 (m, 1H, Im-5H), 7.05-7.35 (m, 5H, Phen-H), 7.81 (s, 1H, Im-2H) ppm.

1-Benzyl-3-(imidazol-4(5)-ylmethyl)pyrrolidine (**22**)

21 Was reduced and hydrolysed, employing the same method as described for the synthesis of **19**. Yield 52%.

^1H ($CDCl_3$) : δ 1.52-2.06 (dm, 2H, Pyrr-4H), 2.06-2.08 (m, 1H, Pyrr-3H), 2.55-2.95 (m, 6H, Pyrr-2H + Pyrr-5H + Im-CH_2), 3.79 (s, 2H, CH_2), 6.63 (s, 1H, Im-5H), 7.17-7.32 (m, 5H, Phen-H), 7.67 (s, 1H, Im-2H) ppm.

^{13}C ($CDCl_3$) : δ 29.49, 30.62, 30.63, 52.57, 57.92, 58.87, 116.98, 128.21, 128.53, 129.56, 132.18, 134.05, 134.06 ppm.

3-(Imidazol-4(5)-ylmethyl)pyrrolidine (VUF 4864) (**23**)

0.3 g (0.12 mmol) **22** was dissolved in 50 ml methanol and 0.1 g 20% $Pd(OH)_2$-C was added carefully. The mixture was stirred for 48 h under hydrogen pressure (35 atm) in an autoclave, filtrated and concentrated *in vacuo*. The residue (0.16 g, 89%) was dissolved in *iso*-propanol and added dropwise to a saturated oxalic acid solution in *iso*-propanol. The precipitate was collected by centrifugation and recrystallized from methanol/ethyl acetate. Melting point 176.5-178.9 °C.

^{1}H NMR (D_2O) : δ 1.61-2.29 (dm, 2H, Pyrr-4H), 2.59-2.79 (m, 1H, Pyrr-3H), 2.79-3.58 (m, 6H, Pyrr-2H + Pyrr-5H + CH_2), 7.27 (s, 1H, Im-4(5)H), 8.58 (s, 1H, Im-2H) ppm.

^{13}C NMR (D_2O) : δ 27.89, 30.79, 38.37, 46.67, 50.78, 117.55, 132.57, 134.74, 168.10 ppm.

Pharmacology

The histamine H_3 activity of the compounds was determined with an *in vitro* assay, based on the inhibitory effect of histamine H_3 agonists on electrically evoked twitches (induced by endogenous acetylcholine release) of guinea pig jejunum preparations (see Chapter 3).[15] The addition of cumulative concentrations of an H_3 agonist, results in a concentration-dependent inhibition of these evoked twitches, from which a concentration-response curve can be constructed. The compounds were tested for H_3 agonism and antagonism. H_3 antagonism was determined against (R)-α-methylhistamine. The potency of the antagonists was expressed by its pA_2 value, calculated from the Schild regression analysis, and at least three different concentrations were used. Statistical analysis was carried out with the Students' *t*-test and $p < 0.05$ was considered statistically significant.

Acknowledgement. We wish to thank Mr. J.P. de Koning and Mr. J.W. Doornenbal for their contribution to the development of the various synthesis routes, Mrs. E. Gelens for the preparation of VUF 4888 and Mrs. S. Gerritsma for the preparation of thioperamide on a large scale, using the described method.

Chapter 10

Cyclopropylhistamine as a Template for an H_3 Receptor Activation Model?

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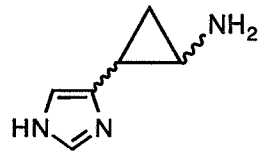
Introduction

For the development of new selective histamine H_3 ligands, it may be useful to construct an activation model for the H_3 receptor, using molecular modelling techniques. Such a three-dimensional model could contribute to a better understanding of the structural requirements of an H_3 agonist and possibly also of an H_3 antagonist. Such an approach might guide us in the optimisation of known H_3 ligands, but might also lead us to the discovery of new lead structures.

Unfortunately, the histamine H_3 receptor has not been cloned yet and not much is known about the receptor topography. Therefore another approach for the identification of the pharmacophore geometry of H_3 ligands has to be used, known as receptor mapping. This method is based on the assumption that the environment of the 'active site' of the receptor is complementary to the structure of the agonists (lock and key principle). Low energy conformations of several potent H_3 agonists can be fitted onto each other and the composite volume of the compounds represents a region in the H_3 receptor, available for the binding of agonists (steric mapping). The addition of inactive compounds or antagonists in this model using the same pharmacophoric groups as fitting points, can indicate regions that can not be accommodated by agonists.
Such a ligand-fit model can also be constructed for H_3 antagonists, but this is more complicated, since antagonists may interact at different sites within the receptor. From the preceding chapters it is clear there is a difference in binding behaviour between different classes of antagonists; compare e.g., the elongated imetit- (Table 16, Chapter 2) and histamine series (Chapter 7) with the burimamide series (Chapter 8). Therefore it appears that there are more binding models for antagonists possible.

A problem for the development of an agonistic model for the histamine H_3 receptor is that potent H_3 agonists, like (R)-α-methylhistamine and imetit can assume a variety of conformations as a result of free rotations about single bonds. Even immepip, with two single bonds and a piperidyl ring, still has a relatively large conformational flexibility. Because most likely only one conformer of an agonist is optimally suited to bind and activate the receptor, there is a need for a small and rigid H_3 agonist to serve as a template for an activation model of the H_3 receptor.

An obvious candidate is cyclopropylhistamine (**1**) (4(5)-(2-aminocyclopropyl)-1*H*-imidazole), which has been reported in a patent application by Arrang *et al.*, to be an agonist with a pD_2 value of 8.0 on rat cortex (see also Chapter 2, table 5).[18]



1, cyclopropylhistamine

Since cyclopropylhistamine (**1**) has two chiral centres and the histamine H_3 receptor is known to be highly stereoselective (see Chapter 2), it seems likely that only one of the four possible stereoisomers is most potent. Unfortunately it was not reported, which of the four possible stereoisomers of cyclopropylhistamine (**1**) is most active.
In the patent application by Arrang *et al.*, describing the agonistic activity of cyclopropylhistamine (**1**),[18] it was mentioned that cyclopropylhistamine had been synthesized according to a method, described by Burger *et al.*[33] This method describes the synthesis of cyclopropylhistamine (**1**), via the cyclopropanation of *sec*-butyl 1-trityl-(*trans*)-urocanate with dimethyloxosulfonium methylide (Corey's reagent),[135] followed by a Curtius rearrangement of the carboxylate group. However, nothing is mentioned about the stereoselectivity of the cyclopropanation reaction, nor about the stereochemistry of the products. Nevertheless, since the dimethyloxosulfonium methylide reacts with the α,β-unsaturated urocanate via a conjugate addition reaction and not by concerted stereospecific addition, the formation of a mixture of isomers is very likely.

Because cyclopropylhistamine (**1**) can serve as a good template for an H_3 receptor activation model, we performed some preliminary calculations in order to predict which stereoisomer might be the most active one. This was done by fitting several potent H_3 agonists onto all four possible stereoisomers of cyclopropylhistamine (**1**) and compare the quality of the fits (best overlap) and the intramolecular energies of the fitted conformations.
From these preliminary calculations, it became clear that there are two distinct possibilities for a model. The first model assumes that histamine (and analogues) is active in the extended conformation ('extended' model). In this model, one of the *trans* isomers of **1** is the most active agonist. The second model assumes a folded active conformer to be the most potent agonist ('folded' model).

For this reason we subsequently decided to develop a route for the synthesis of *trans*-cyclopropylhistamine (*trans*-**1**) and *cis*-cyclopropylhistamine (*cis*-**1**).

Results and Discussion

Preliminary Modelling

It was assumed that in solution at physiological pH; cyclopropylhistamine (**1**) is predominantly present in its monocationic form, just as calculated for histamine,[136] (R)-α-methylhistamine[137] and (R)α,(S)β-dimethylhistamine.[138] Therefore, the lowest energy conformations for the four stereoisomers of the monocationic species of cyclopropylhistamine (**1**), were determined with the conformational analysis tools in MACROMODEL, using the Amber force field[139] (see Figure 1).

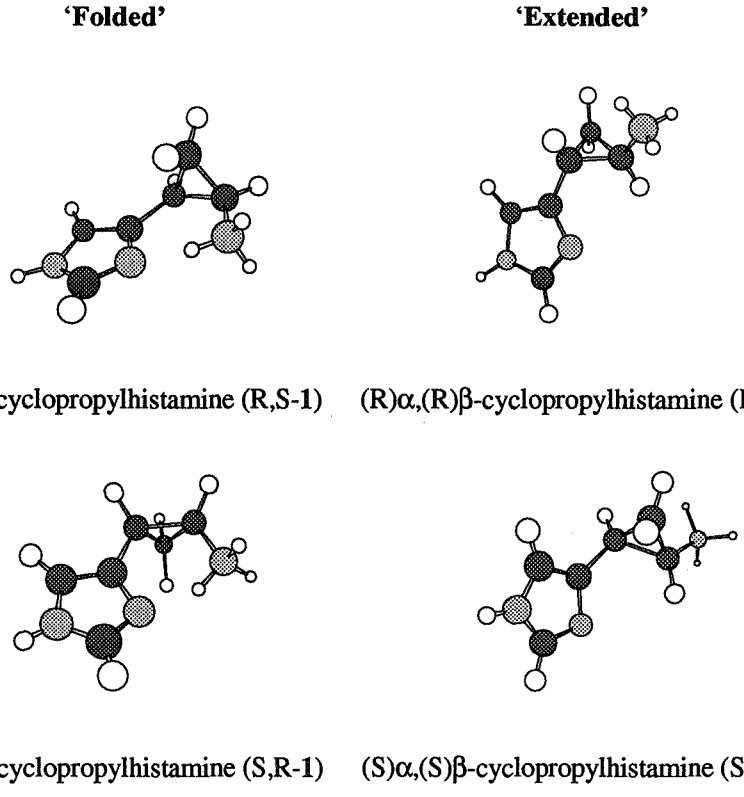
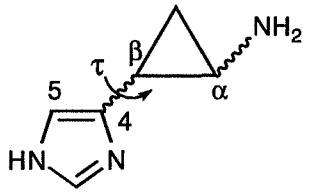


Figure 1 Lowest energy conformations of the four stereoisomers of cyclopropylhistamine (**1**) as calculated with MACROMODEL using the Amber force field.

The four stereoisomers were further optimized with the semi-empirical MNDO-calculation method[140] (MOPAC, version 6.0). Comparable torsion angles were observed for the lowest energy conformations.

Table 1. Torsion angles of the four optimized stereoisomers of **1**, as calculated with molecular mechanics (AMBER) and semi-empirical methods (MNDO).



Compound		AMBER		MNDO
		E (Kcal/mol)	τ^a	τ^a
cis-**1**	R,S-**1**	95	-177	-171
"	S,R-**1**	95	177	180
trans-**1**	R,R-**1**	104	-142	-150
"	S,S,-**1**	104	142	135

[a] The torsional angle is defined by : C_5-C_4-C_β-C_α.

From the results it was obvious that there are two distinct possibilities:

1) In the monocationic form, (R)α,(S)β-cyclopropylhistamine (R,S-**1**) and (S)α,(R)β-cyclopropylhistamine (S,R-**1**) (both *cis*-enantiomers) forms an internal hydrogen-bridge between the cationic primary amine and the N^π of the imidazole ring, which will be favourable in solution. If one of these two enantiomers is the most potent H_3 agonist of the four possible isomers, a 'folded' model is a strong possibility, where internal hydrogen bridge formation is important for activity.
2) If one of the two *trans*-enantiomers has the highest H_3 activity, of the four possible stereoisomers, an 'extended' model is favourable and no internal hydrogen bridge is required for H_3 agonism.

The global energy minima of the four stereoisomers, in a monocationic form were used as a template for further ligand-fit procedures. Conformational analyses were performed on a few potent H_3 agonists, like (R)-α-methylhistamine, (R)α,(S)β-dimethylhistamine and immepip. The lowest energy conformations of these agonists were fitted on the most stable conformations of **1**, using CHEMX90. Within the fitting procedures, a restraint constant of 50 Kcal/mol/Å^2 was used for the nitrogen atoms and the C-2 atoms in the imidazole rings to be matched. The nitrogen atoms of the side chains were fitted with a restrain constant of 100 Kcal/mol/Å^2 in a following flexible fitting procedure.

Two main criteria were used to determine which of the four templates shows the 'best fit' and is therefore likely to be the most active histamine H_3 agonist.
The energy difference between the conformation of the fitted agonist and its global energy conformation had to be low (<5 Kcal/mol), since it is unlikely that the agonist will assume a high energy conformation. Furthermore it was assumed that there should be a large steric overlap of the different agonists. Therefore the excluded Van der Waal's volume,* with respect to the template, was calculated. The smaller the excluded volume, the better the fit.

Table 2. Intramolecular energy differences (Δ E) and excluded volumes (Δ V) for the conformations of a few H_3 agonists, fitted onto the different isomers of **1**.

	MNDO				Amber	
	(R)α,(S)β-diMeHa [a]		(R)-α-MeHa [a]		immepip	
Comp.	Δ E (Kcal/mol)	Δ V (Å)	Δ E (Kcal/mol)	Δ V (Å)	Δ E (Kcal/mol)	Δ V (Å)
R,S-1	0.9	32.4	1.3	31.4	8.6	41.4
S,R-1	1.0	38.3	0.7	38.9		
R,R-1	13.2	56.1	18.8	47.7	17.9	44.4
S,S-1	8.8	39.6	14.6	40.0		

[a] MeHa = methylhistamine.

* Common volume of template and fitted compound, minus the volume of the template alone.

The results suggest that the H_3 agonists fitted best on (R)α,(S)β-cyclopropylhistamine (R,S-**1**) (best volume overlap and small energy differences between ground state and fitted states). These findings indicate that (R)α,(S)β-cyclopropylhistamine (R,S-**1**) is an active H_3 agonist and that an internal hydrogen bridge might be important for H_3 agonism.

This suggestion is in agreement with recent calculations on histamine and (R)α,(S)β-dimethylhistamine, in aqueous solution, by Nagy *et al.*[138] Calculations from this group predicted the gauche conformer of histamine and (R)α,(S)β-dimethylhistamine to be the predominant species in solution, indicating that hydrogen bonding is commonly observed for H_3 agonists. Also other reports underline the importance of an intramolecular hydrogen bond for H_3 agonists.[137]

This could also be an explanation for the finding that immepip and imetit are potent H_3 agonists as well, because the internal hydrogen bridge positions the side chain nitrogen atoms of immepip and imetit in a comparable position relative to the imidazole ring, as with the histamine analogues. Immepip fits the 'folded' model in a 'boat' conformation.

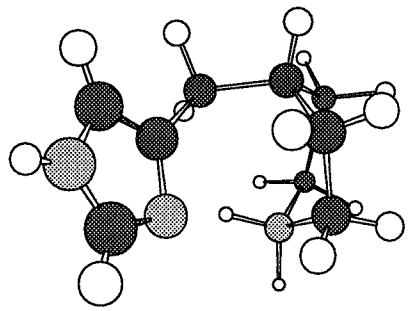


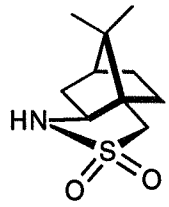
Figure 2 Immepip in a 'folded' conformation.

From our preliminary modelling results, it appears that a 'folded' model is most likely, but this conclusion is still highly speculative. Therefore the synthesis of the *trans*- and the *cis*-diastereoisomers of cyclopropylhistamine becomes very important for the development of an H_3 agonistic receptor model.

Synthesis

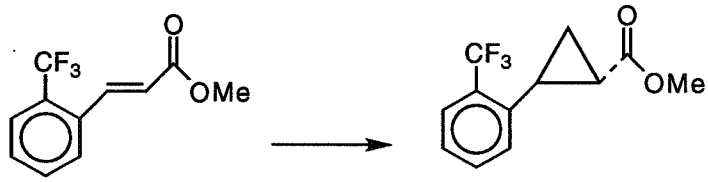
trans-*cyclopropylhistamine*

The key step in the synthesis route of *trans*-**1** is the cyclopropanation[141] of the methyl 1-protected-urocanate **4** by the stereospecific addition of diazomethane, using palladium(II)acetate as a catalyst. This method has been described as highly stereoselective for the cyclopropanation of α,β-unsaturated carboxylic acids, derivatized with camphorsultam[142,143] as a chiral auxiliary.[144,145]

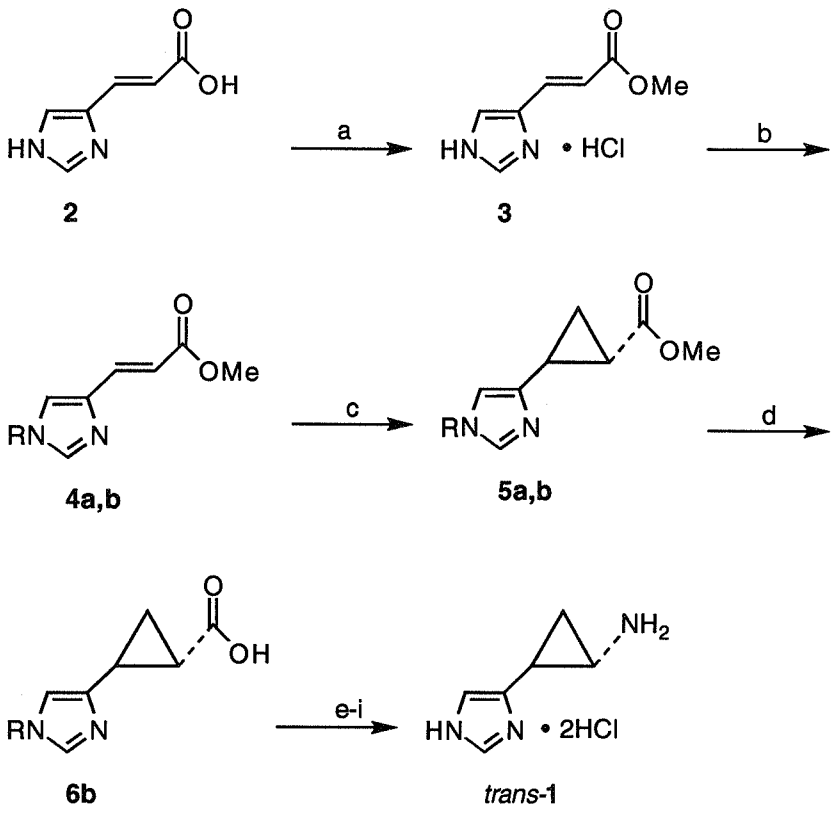


camphor sultam (bornan-10,2-sultam)

This approach was applied successfully in the quantitative cyclopropanation of e.g., methyl (*trans*)-3-[2-(trifluoromethyl)phenyl]acrylate.[145]



For the synthesis of *trans*-cylcopropylhistamine, the readily available (*trans*), urocanic acid (**2**) was used as starting material.



a; R = *p*-$CH_3C_6H_5SO_2$-,

b; R = $(CH_3)_2NSO_2$-

Scheme 2 Reagents used : (a) HCl-gas, MeOH, reflux; (b) R-Cl, Et_3N, DCM; (c) CH_2N_2, cat. $Pd(OAc)_2$, DCM; (d) 1M KOH, MeOH, THF; (e) EtCOCl, Et_3N, acetone; (f) NaN_3, H_2O; (g) toluene, reflux; (h) tert-butanol, reflux; (i) 1M HCl, reflux.

The carboxylic acid function of urocanic acid (**2**) was protected by esterification in methanol, with HCl-gas, leading to **3**.

Initially we used the *p*-tosyl group for the protection of the imidazole and cyclopropanation of **4a**, with diazomethane and palladium(II)acetate as a catalyst, proved possible. It must be noted however, that higher temperatures and larger amounts of catalyst were required than described for e.g. the cyclopropanation shown in scheme 1.

Unfortunately, hydrolysis of ester **5a** under various basic conditions, to obtain the carboxylic acid derivative **6**, resulted in the hydrolysis of the *p*-tosyl group as well. Hydrolysis of ester **5a** under acidic conditions was also not successful, since only deprotection of the imidazole ring was observed and no ester hydrolysis. Dealkylation of the ester (**5a**) with a chlorotrimethylsilane-sodium iodide complex[146] did not lead to **6a** either.

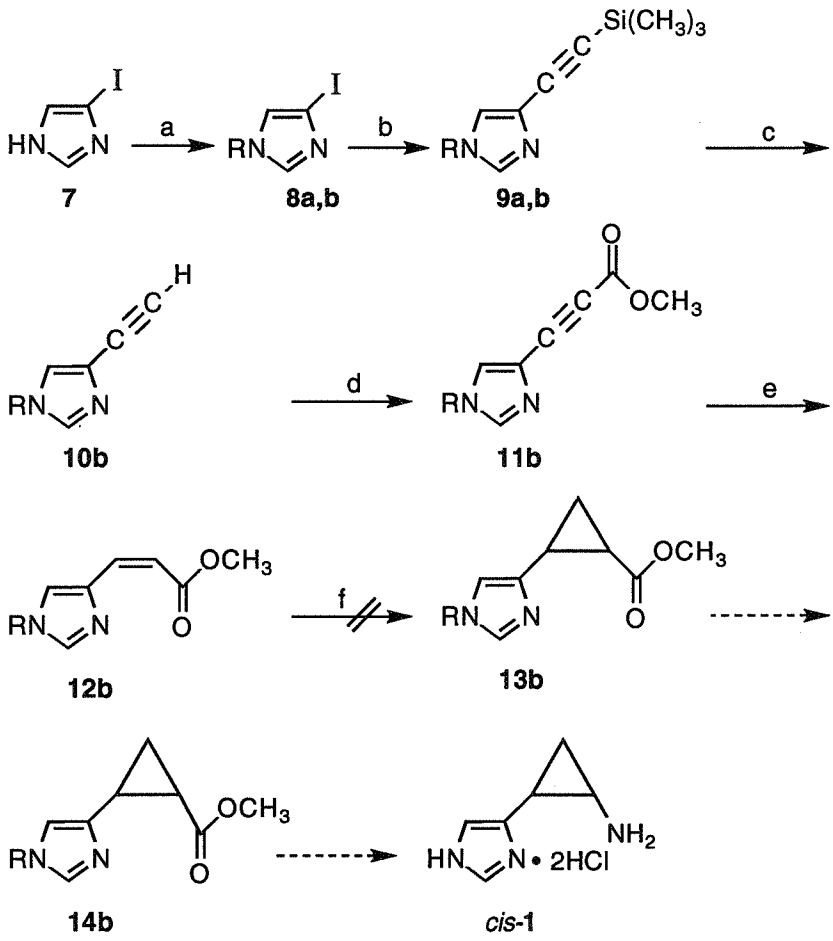
Because the *p*-tosyl group was obviously not 'perfect' for our reaction sequence, another protecting group for the NH function of the imidazole ring was selected. The *N,N*-dimethylsulfamoyl group proved to be more suitable, since cyclopropanation of **4b** was successful and hydrolysis of **5b** to **6b** proceeded readily.

The carboxylic acid derivative **6b** was subsequently converted in *trans*-cyclopropylhistamine (*trans*-**1**) via a Curtius rearrangement. The resultant isocyanate derivative was treated with *t*-butanol to give the carbamate, which was subsequently hydrolysed under acidic conditions, causing the removal of the *N,N*-dimethylsulfamoyl group as well. The overall yield of this synthesis route, starting from urocanic acid (**2**) was 30%.

cis-*cyclopropylhistamine*

For the synthesis of the *cis*-diastereoisomer of cyclopropylhistamine (*cis*-**1**), we tried to use a comparable approach as for the synthesis of *trans*-**1**. Therefore the methyl 1-protected-(*cis*)-urocanate (**12**) had to be prepared. For the synthesis of this compound, we developed a route, based on the coupling of trimethylsilylacetylene to a 1-protected-4-iodoimidazole (**8**).

It was reported in literature that acetylenic hydrogen atoms can be readily substituted by aryl halides in the presence of the copper(I)iodide/bis[triphenylphosphine]-palladium dichloride-complex as a catalyst in amines as solvent.[147] For the synthesis of terminal acetylenic compounds, the reaction was performed with trimethylsilylacetylene and it was reported that trimethylsilyl group was removed quantitatively by basic hydrolysis.[148]



a; R = *p*-$CH_3C_6H_5SO_2$-,

b; R = $(CH_3)_2NSO_2$-

Scheme 3 Reagents used : (a) R-Cl, Et_3N, toluene or DCM; (b) trimethylsilylacetylene, cat. $[(C_6H_5)_3P]_2PdCl_2$, cat. CuI, Et_3N, 50°C; (c) 2M KOH, MeOH, THF; (d) EtMgBr, $(CH_3)_2CO$; (e) H_2-gas, cat. Lindlar, cat. quinoline, acetone; (f) CH_2N_2, cat. $Pd(OAc)_2$, DCM.

We prepared 4-iodoimidazole (**7**) and protected the imidazole initially with a *p*-tosyl group (**8a**). The iodo atom of compound (**8a**), was readily substituted by the trimethylsilylacetylene group, leading to **9a**. Unfortunately, attempts to hydrolyse the trimethylsilyl group of **9a** under various basic conditions, were not satisfactory, because the *p*-tosyl group was hydrolysed as well. Also treatment with tetrabutylammonium fluoride was unsuccessful. Again the use of the *N,N*-dimethylsulfamoyl

group as an imidazole protecting group proved to be more satisfying. After the palladium catalysed coupling reaction of **8b** with trimethylsilylacetylene, **9b** was easily hydrolysed to **10b**.
The conversion of the terminal acetylenic derivative **10b** in alkynester **11b** was initially tried by oxidative carbonylation in methanol, with carbon monoxide in the presence of palladium(II)chloride and copper(II)chloride.[149] No reaction was observed, but the treatment of **10b** with ethylmagnesium bromide and dimethylcarbonate was successful and resulted in **11b**.
The alkynester **11b** was reduced to the *cis*-alkene **12b**, by hydrogenation under atmospheric pressure in the presence of Lindlar catalyst (palladium on calcium carbonate, poisoned with lead).

The cyclopropanation of **12b** to compound **13b** proved to be more troublesome. All attempts to perform this reaction under the same, or even more drastic, conditions as for the *trans*-alkene ester **4b**, were unsuccessful, although the alkene moiety seems sterically rather accessible. Apparently the *cis*-alkene **12b** is seriously deactivated towards attack by diazomethane. Deactivation of the catalyst ($Pd(OAc)_2$ by chelation might be another explanation for these findings.

Pharmacology

The H_3 activity of *trans*-cyclopropylhistamine (*trans*-**1**), was determined on an *in vitro* test system, based on the concentration-dependent inhibitory effect of histamine H_3 agonists on the electrically evoked contractile response of isolated guinea pig jejunum segments (see Chapter 3).[15]
This compound proved to be only a weak partial H_3 agonist on this test system.

$$pD_2 = 6.5 \pm 0.1; \text{ intrinsic activity} = 0.3$$

$$pA_2 = 6.2 \pm 0.1; \text{ Schild-slope} = 0.8 \pm 0.1$$

Data expressed as mean ± SD Three different animals used. Slope of Schild plot not significantly different from unity.
The H_3 agonistic activity could not be verified by the determination of the pA_2 value of a selective H_3 antagonist (e.g. thioperamide), using *trans*-**1** as agonist, because of its very low intrinsic activity.

This observation is clearly different from the high pD_2 value of 8.0 on rat cortex, reported by Arrang *et al.*[18] and could be an indication that the material as tested by this group does not consist of the pure *trans*-isomer, but likely a mixture of *cis* and *trans*-cyclopropylhistamine. Therefore, one of the enantiomers of *cis*-cyclopropylhistamine (*cis*-**1**) could be responsible for the high agonistic properties of the unresolved mixture as reported by the authors.

Conclusions

Obviously, cyclopropylhistamine (**1**) can be a valuable tool for the development of an agonistic H_3 receptor model, but the stereoselective synthesis and the determination of the most active stereoisomer of **1** is required.
trans-Cyclopropylhistamine proved to be only very weakly active. Assuming that the reported potent agonistic activity in the patent literature,[18] is reliable, it is likely that one of the *cis*-enantiomers of **1** is active and preparation of *cis*-**1** is required. However, in case the actual activity of the isomeric mixture as published in the patent application is actively much lower than reported, such an observation might be used in the reversed sense: certain conformations can be excluded for activity.
Unfortunately the cyclopropanation of the *cis*-alkene **12b** with diazomethane in the presence of a palladium catalyst, as successfully applied in the synthesis of *trans*-**1**, has failed thusfar. It might be useful to try a different cyclopropanation reaction with **12b**. Another option might be the exchange of the *N,N*-dimethylsulfamoyl group with another imidazole protecting group.
If (R)α,(S)β-cyclopropylhistamine (R,S-1) (best calculated fit) is indeed the most active stereoisomer of **1** it seems likely that an internal hydrogen bond is essential for the H_3 agonistic effect of histamine analogues.

Experimental Section

^1H NMR and ^{13}C NMR spectra were recorded on JEOL JNM-EX270. GLC-Analysis was performed on a Shimadzu GC-14A equipped with an FID detector and an HP1 column (50 m x 0.32 mm). Melting points were determined in open glass capillaries on a Thomas-Hoover apparatus and are uncorrected. Elemental analyses was performed by Micro Kemi AB, Uppsala, Sweden.
THF was distilled from $LiAlH_4$ and dimethylcarbonate was dried overnight on molecular sieves (4Å).
4(5)-Iodoimidazole (**7**) was prepared by the reduction of 4,5-di-iodoimidazole with 3 eq Na_2SO_3 as described by Pauly *et al.*[150,151] 4,5-Di-iodoimidazole was prepared by the addition of a solution of iodine in 20% potassium iodide to a stirred solution of imidazole in 2M sodium hydroxide. This was reported by Iddon and Lim[106] for the synthesis of 2,4,5-tri-iodoimidazole, but in our hands this reaction results only in 4,5-di-iodoimidazole, as was noted by Turner and Lindell[101] as well.

Methyl (trans)-*3-(imidazol-4(5)-yl)acrylate hydrochloride* (**3**)

25.0 g (0.18 mol) Urocanic acid (**2**) was added to 150 ml refluxing methanol. HCl-gas was bubbled through the mixture, according to literature procedures.[152] After 5 h of refluxing, the clear solution was cooled down to 5 °C and white needle-like crystals appeared. Yield : 32.20 g (94.4%). Melting point 233.5 - 234.5 °C.

Methyl (trans)-*3-[1-*(p-*toluenesulfonyl)imidazol-4-yl]acrylate* (**4a**)

10.0 g (53 mmol) **3** Was dissolved in a solution of 400 ml dichloromethane and 50 ml triethylamine. 11.0 g (58 mmol) *p*-Tosylchloride was added and the reaction mixture stirred at room temperature for 16 h. The solution was washed with 150 ml H_2O and 150 ml Brine. After evaporation of the dichloromethane, 16.72 g of a white solid remained. After recrystallization from methanol, 15.05 g of white cotton-like crystals where collected (92.6%). Melting point 161.0 - 162.5 °C.

^1H NMR ($CDCl_3$) : δ 2.45 (s, 3H, Tol-CH_3), 3.77 (s, 3H, OCH_3), 6.62 (d, 1H, J 15.6 Hz, =CH), 7.38 (d, 2H, J 8.5 Hz, 2 x phenyl-H), 7.39 (s, 1H, Im-5H), 7.44 (d, 1H, J 15.6 Hz, =CH), 7.84 (d, 1H, J 8.3 Hz, 2 x phenyl-H), 7.99 (s, 1H, Im-2H) ppm.

^{13}C NMR ($CDCl_3$) : δ 21.8, 51.7, 118.1, 119.4, 127.5, 130.6, 134.2, 134.4, 137.4, 140.2, 146.8, 167.2 ppm.

Anal. ($C_{14}H_{14}N_2O_4S$) : 54.9 %C, 4.5 %H, 9.1 %N;
Calcd. : 54.9 %C, 4.6 %H, 9.1 %N.

Methyl (trans)-*2-[1-*(p-*toluenesulfonyl)imidazol-4-yl]cyclopropanecarboxylate* (**5a**)

A solution of 52.5 g (245 mmol) *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (Diazogen) dissolved in 500 ml ether was added dropwise to a heated mixture of 41.0 g (730 mmol) KOH in 150 ml H_2O and 150 ml 2-(2-ethoxyethoxy)ethanol. Special 'soft' glassware was used. The hence generated etheric diazomethane solution was distilled dropwise in a solution of 5.0 g (16 mmol) **4a** in 1 L dichloromethane, together with 150 mg $Pd(OAc)_2$ at room temperature. The light yellow solution became dark yellow and N_2-evolution was observed. The reaction mixture stirred overnight. After the addition of a few drops acetic acid, the reaction mixture was washed with 250 ml 5% $NaHCO_3$-solution. The organic layer was dried on Na_2SO_4, filtrated over a short silica column and concentrated *in vacuo*. The remaining light yellow oil consisted of 74 % product and 26 % starting material according to GC and ^1H NMR. The residue was extracted with methanol (starting material dissolves only slightly in methanol), concentrated *in vacuo* and recrystallized from *n*-hexane. A white solid (4.30 g) was isolated, which still contained 10% **4a**.

^1H NMR ($CDCl_3$) : δ 1.37-1.52 (m, 2H, CH_2), 1.98-2.04 (m, 1H, CH), 2.33-2.40 (m, 1H, CH), 244 (s, 3H, Tol-CH_3), 3.68 (s, 3H, OCH_3), 7.08 (s, 1H, Im-5H), 7.36 (d, 2H, J 8.1 Hz, 2 x phenyl-H), 7.81 (d, 1H, J 8.4 Hz, 2 x phenyl-H), 7.85 (s, 1H, Im-2H) ppm.

Methyl (trans)-*3-[1-*(N,N-*dimethylsulfamoyl)imidazol-4-yl]acrylate* (**4b**)

32.20 g (0.17 mol) **3** Was dissolved in a solution of 600 ml dichloromethane and 75 ml triethylamine. After the addition of 20.0 ml (0.19 mol) *N,N*-dimethylsulfamoyl-chloride, the reaction mixture was refluxed for 48 h. The solution was washed with 150 ml H_2O and 150 ml Brine. After evaporation of the dichloromethane, 43.37 g of a white solid remained. Recrystallization from *iso*-propanol resulted in 37.55 g (84.8%) white crystals. Melting point 137.0-138.0 °C.

^1H NMR ($CDCl_3$) : δ 2.89 (s, 6H, NCH_3), 3.80 (s, 3H, OCH_3), 6.67 (d, 1H, J 15.6 Hz, =CH), 7.38 (s, 1H, Im-5H), 7.52 (d, 1H, J 15.6 Hz, =CH), 7.90 Im-2H) ppm.

^{13}C NMR ($CDCl_3$) : δ 38.2, 51.7, 118.7, 119.1, 134.4, 137.5, 139.3, 167.3 ppm.

Anal. ($C_9H_{13}N_3O_4S$) : 41.7 %C, 5.2 %H, 16.6 %N;
Calcd. : 41.7 %C, 5.1 %H, 16.2 %N.

(trans)-*2-[1-(*N,N-*Dimethylsulfamoyl)imidazol-4-yl]cyclopropanecarboxylic acid* (**6b**)

A solution of 40.0 g (0.19 mol) *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (Diazogen) dissolved in 200 ml ether was added dropwise to a heated mixture of 31.0 g (0.55 mol) KOH in 200 ml H_2O and 200 ml 2-(2-ethoxyethoxy)ethanol. Special 'soft' glassware was used. The hence generated etheric diazomethane solution was distilled dropwise in a solution of 5.0 g (19.3 mmol) **4b** in 1 L dichloromethane, together with 200 mg $Pd(OAc)_2$ at room temperature. The light yellow solution became dark brown and N_2-evolution was observed. The reaction mixture stirred overnight. After the addition of a few drops acetic acid, the reaction mixture was washed with 250 ml 5% $NaHCO_3$-solution. The organic layer was dried on Na_2SO_4, filtrated over a short silica column and concentrated *in vacuo*. The remaining light yellow oil consisted of 49 % product and 51 % starting material according to GC and ^1H NMR.

^1H NMR ($CDCl_3$) (**5b**) : δ 1.42-1.57 (m, 2H, CH_2), 2.03-2.13 (m, 1H, CH), 2.30-2.48 (m, 1H, CH), 284 (s, 6H, NCH_3), 3.70 (s, 3H, OCH_3), 7.08 (s, 1H, Im-5H), 7.77 (s, 1H, Im-2H) ppm.

The residue was dissolved in 1 L dichloromethane and the procedure was repeated, employing the same amounts of reagents. This time the residing oil consisted of 82 % product and was dissolved in a mixture of 100 ml methanol and 100 ml tetrahydrofuran. 100 ml 1M KOH-solution was added and after 0.5 h stirring at room temperature, the mixture was washed with dichloromethane (3 x 200 ml). The water-layer was treated with 1M HCl-solution until the solution had a pH of 2. The product was extracted with dichloromethane (3 x 200 ml) and after drying and evaporation of the dichloromethane, 3.91 g of a light brown solidified oil remained. After several triturations with dry acetone, 2.92 g (58.4%) of a white powder was collected. Melting point 166.0 °C.

^1H NMR (DMSO-d_6) (**6b**) : δ 1.28-1.37 (m, 2H, CH_2), 1.78-1.88 (m, 1H, CH), 2.28-2.38 (m, 1H, CH), 2.78 (s, 6H, CH_3), 7.50 (s, 1H, Im-5H), 8.00 (s, 1H, Im-2H), 12.28 (bs, 1H, CO_2H) ppm.

^{13}C NMR (DMSO-d_6) (**6b**) : δ 14.8, 18.7, 22.0, 37.7, 114.1, 136.7, 141.5, 173.8 ppm.
Anal. ($C_9H_{13}N_3O_4S$) : 41.5 %C, 4.9 %H, 16.2 %N;
Calcd. : 41.7 %C, 5.1 %H, 16.2 %N.

(trans)-*2-(Imidazol-4(5)-yl)cyclopropanamine* (*trans*-**1**)

2.22 g (8.6 mmol) **6b** was dissolved in 65 ml dry acetone and 1.6 ml (11.5 mmol) triethylamine under N_2 atmosphere. The reaction mixture was cooled on a 0 °C bath and 1.6 ml (16.7 mmol) ethylchoroformate was added dropwise (a white precipitate formed). After 2 h, a solution of 0.85 g (13.1 mmol) sodiumazide in 15 ml H_2O was added slowly. The mixture was stirred for an additional hour. After addition of 65 ml H_2O, the solution was concentrated *in vacuo* until the water-layer remained. The water-layer was extracted with toluene (3 x 65 ml) and the toluene-layers were combined, dried on Na_2SO_4 and refluxed for 2 h (N_2-evolution was observed). The residue after evaporation of toluene was refluxed in 50 ml *tert*-butanol for 12 h (no isocyanate-peak in IR-spectrum). After evaporation of the alcohol a dark residue remained. This residue was dissolved in ethyl acetate and filtrated over a short silica column. 2.10 g of a white solid remained after evaporation of the ethyl acetate. This was refluxed for 12 h in 100 ml 1M HCl. After concentration *in vacuo*, the residue was refluxed in abs. ethanol for 0.5 h and subsequently washed with acetone. 1.06 g (63%; 30% overall, starting from urocanic acid (**2**)) Of a light beige solid remained, which was recrystallized from *iso*-propanol/ether. Melting point 187.0-187.5 °C.

^{1}H NMR (D_2O) : δ 1.42 (ddd, 1H, J 6.7, 6.9 and 8.2 Hz), 1.55 (ddd, 1H, J 4.7, 7.2 and 10.2 Hz), 2.51 (ddd, 1H, J 3.4, 6.5 and 10.1 Hz), 3.03 (ddd, 1H, J 3.6, 4.5 and 8.2 Hz), 7.25 (s, 1H, Im-4(5)H), 8.57 (s, 1H, Im-2H) ppm.

^{13}C NMR (D_2O) : δ 12.4, 12.5, 30.7, 117.4, 132.4, 134.9 ppm.
Anal. ($C_6H_9N_3$•2HCl) : 36.8 %C, 5.5%H, 21.5 %N;
Calcd. : 36.8 %C, 5.7 %H, 21.4 %N.

*1-(*p*-Toluenesulfonyl)-4-iodoimidazole* (**8a**)

10.0 g (52 mmol) *p*-Tosylchloride was added to a stirred solution of 10.0 g (52 mmol) 4(5)-Iodoimidazole (**7**) in 500 ml toluene and 100 ml triethylamine. After 16 h, the reaction mixture was filtrated and concentrated *in vacuo*. The white residue (19.20 g) was recrystallized from methanol and 13.00 g (72%) of white crystals where collected. Melting point 156 °C.

^{1}H NMR ($CDCl_3$) : δ 2.46 (s, 3H, Tol-CH_3), 7.36 (s, 1H, Im-5H), 7.38 (d, 2H, J 9.0 Hz, phenyl-H), 7.82 (d, 2H, 10.0Hz), phenyl-H), 7.87 (s, 1H, Im-2H) ppm.

^{13}C NMR ($CDCl_3$) : δ 21.8, 85.2, 122.4, 127.6, 130.6, 134.2, 137.7, 146.9 ppm.

Anal. ($C_{10}H_9N_2O_2SI$•2HCl) : 34.6 %C, 2.4 %H, 8.0 %N;
Calcd. : 34.5 %C, 2.6 %H, 8.0 %N.

1-(p-Toluenesulfonyl)-4-[(1-trimethylsilyl)ethyn-2-yl]imidazole (**9a**)

6.0 g (17 mmol) **8a** was stirred in 200 ml triethylamine under N_2-atmosphere. To this mixture 120 mg (1%) bis(triphenylphosphine)palladium(II)chloride, 35 mg CuI (1%) and 5.0 ml trimethylsilylacetylene (2 eq) was added. This was stirred for 12 h on a 50 °C oil bath. The brown mixture was filtrated and the filtrate was concentrated *in vacuo*. The brown solid was washed several times with *n*-hexane and a white solid was collected.

^{1}H NMR ($CDCl_3$) : δ 0.21 (s, 9H, $SiCH_3$), 2.45 (s, 3H, Tol-CH_3), 7.36 (d, 2H, phenyl-H), 7.41 (s, 1H, Im-5H), 7.81 (d, 2H, phenyl-H), 7.91 (s, 1H, Im-2H) ppm.

1-(N,N,-Dimethylsulfamoyl)-4-iodoimidazole (**8b**)

12.0 ml (0.11 mol) *N,N*-Dimethylsulfamoylchoride was added to a solution of 20.0 g (0.10 mol) 4-iodoimidazole (**7**) in 600 ml dichloromethane and 40 ml triethylamine. The reaction mixture was refluxed for 60 h and subsequently washed with 250 ml H_2O and 250 ml $Na_2S_2O_3$-solution (5%). 30.22 g Of a light brown solid remained after evaporation of the solvents. Recrystallization from hot *iso*-propanol resulted in 18.65 g (60 %) white crystals. Melting point 131.5 °C.

^{1}H NMR ($CDCl_3$) : δ 2.89 (s, 6H, CH_3), 7.34 (s, 1H, Im-5H), 7.78 (s, 1H, Im-2H) ppm.

^{13}C NMR ($CDCl_3$) : δ 38.2, 84.4, 122.7, 137.8 ppm.
Anal. ($C_5H_8N_3O_2SI$) : 19.8 %C, 2.5 %H, 14.0 %N;
Calcd. : 19.9 %C, 2.7 %H, 14.0 %N.

1-(N,N-Dimethylsulfamoyl)-4-[3-(ethyl 2-propynoate)]imidazole (**11b**)

20.0 g (67 mmol) **8b** was stirred in 250 ml triethylamine under N_2-atmosphere. To this mixture 0.5 g bis(triphenylphosphine)palladium(II)chloride (1 mol%), 130 mg CuI (1 mol%) and 20.0 ml trimethylsilylacetylene (141 mmol) was added. This was stirred for 60 h on a 50 °C oil bath. After filtration of the reaction mixture, the solution was concentrated *in vacuo*.

^{1}H NMR ($CDCl_3$) (**9b**) : δ 0.25 (s, 9H, $SiCH_3$), 2.87 (s, 6H, NCH_3), 7.40 (s, 1H, Im-5H), 7.81 (s, 1H, Im-2H) ppm.

The residue (**9b**) was dissolved in 150 ml methanol and 150 ml tetrahydrofuran. 150 ml 2M potassium hydroxide was added and the mixture was poured in 150 ml of a saturated NH_4Cl-solution. After extraction of the product with dichloromethane (1 x 300 ml; 2 x 100 ml), drying on Na_2SO_4 and evaporation of the dichloromethane, 12.30 g of a brown oil remained. The oil was purified on a silicagel column with ethyl acetate as eluent(R_f = 0.7). 7.82 g (59%) of a light beige solid was isolated.

^1H NMR ($CDCl_3$) (**10b**) : δ 2.88 (s, 6H, NCH_3), 3.13 (s, 1H, CH), 7.42 (s, 1H, Im-5H), 7.83 (s, 1H, Im-2H) ppm.

5.0 g (25 mmol) of this beige solid (**10b**) was dissolved in 50 ml dichloromethane and added dropwise to a Grignard mixture, prepared with 0.8 g (33 mmol) magnesium and 2.5 ml (33 mmol) ethyl bromide in 5 ml tetrahydrofuran. After 1 h the mixture was poured in 100 ml dimethylcarbonate and stirred overnight. The solution was poured in 250 ml H_2O and extracted with dichloromethane (3 x 200 ml). After evaporation of the solvents, 5.53 g of a brow oil remained, which was purified on a silicagel column with ethyl acetate as eluent. (R_f = 0.8). After two Recrystallization from *iso*-propanol, 2.98 g (46%) white crystals where collected. Melting point 126.0-127.0 °C.

^1H NMR ($CDCl_3$) (**11b**) : δ 2.91 (s, 6H, NCH_3), 3.84 (s, 3H, OCH_3), 7.63 (s, 1H, Im-5H), 7.88 (s, 1H, Im-2H) ppm.

^{13}C NMR ($CDCl_3$) (**11b**) : δ 38.2, 52.9, 78.6, 82.1, 123.2, 124.6, 137.1, 154.0 ppm.
Anal. ($C_9H_{11}N_3O_4S$) : 41.7 %C, 4.1 %H, 16.3 %N;
Calcd. : 42.0 %C, 4.3 %H, 16.3 %N.

Methyl (cis)-*3-[1-*(N,N-*dimethylsulfamoyl)imidazol-4-yl]acrylate* (**12b**)

2.6 g (10.1 mmol) **11b** was dissolved in 75 ml acetone. 120 mg Lindlar catalyst and 300 mg quinoline were added and the reaction mixture was stirred under hydrogen-atmosphere (1 atm). After hydrogen consumption had ceased, additional Lindlar catalyst was added (repeated twice; a total of 280 mg catalyst extra was added). After 8 h and 300 ml of hydrogen gas consumption, the reaction mixture was filtrated and concentrated *in vacuo*. The residue consisted of 92% product and 8% of the *trans* isomer, according to GC. This was purified on a silicagel column with ethyl acetate as eluent (R_f = 0.5) and recrystallization from *n*-hexane. 1.8 g (69%) white crystals where isolated. Melting point 74.0 °C.

^1H NMR ($CDCl_3$) : δ 2.92 (s, 6H, NCH_3), 3.76 (s, 3H, OCH_3), 5.95 (d, 1H, J 12.7Hz, =CH), 6.96 (d, 1H, J 12.2Hz, =CH), 7.88 (s, 1H, Im-5H), 8.64 (s, 1H, Im-2H) ppm.

Anal. ($C_9H_{13}N_3O_4S$) : 41.6 %C, 5.0 %H, 16.4 %N;
Calcd. : 41.7 %C, 5.0 %H, 16.2 %N.

Pharmacology

The histamine H_3 activity of the compounds was determined on an *in vitro* assay, based on the inhibitory effect of histamine H_3 agonists on electrically evoked twitches (induced by endogenous acetylcholine release) of guinea pig jejunum preparations (see Chapter 3).[15] The addition of cumulative concentrations of an H_3 agonist, results in a concentration-dependent inhibition of these evoked twitches, from which a concentration-response curve can be constructed. The compounds were tested for H_3 agonism and antagonism. H_3 antagonism was determined against (R)-α-methylhistamine. The potency of the antagonists was expressed by its pA_2 value, calculated from the Schild regression analysis, and at least three different concentrations were used. Statistical analysis was carried out with the Student' *t*-test and $p < 0.05$ was considered statistically significant.

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Concluding Remarks

With the development of a new route for the synthesis of a variety of 4(5)-substituted imidazoles, it was possible to obtain several new ligands of the histamine H_3 receptor.

Especially remarkable are the structurally new potent and selective H_3 agonist immepip (Chapter 6), and the H_3 antagonist impentamine (Chapter 7). Both compounds can be useful as pharmacological tools and perhaps in the future as therapeutical agents. The histamine H_3 receptor currently attracts interest from industries, for the development of therapies for e.g. epilepsy, Alzheimer's disease and sleeping-disorders. For these applications H_3 receptor antagonists might be useful.

For the design of an agonistic model for the histamine H_3 receptor, the small and rigid cyclopropylhistamine (Chapter 10) might serve as a template, but isolation and determination of the activity of the pure stereoisomers is required. Immepip (and its pyrrolidine derivative VUF 4864) (Chapter 9) can also be very useful in the development of an H_3 receptor activation model, because of its rigid structure, different from histamine and its derivatives.

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Summary

The research described in this thesis was mainly focused on the synthesis and development of new ligands for the histamine H_3 receptor, as tools for molecular pharmacology- and structure activity relationship studies. This was mainly accomplished by structural modifications of some well known histamine H_3 ligands.
An overview of all the reported compounds that are active on the histamine H_3 receptor, either as agonists or as antagonists, is presented in chapter 2.
The H_3 activity of novel synthesized compounds, described in this thesis, was determined on a specifically developed *in vitro* assay for the rapid screening of histamine H_3 ligands. This simple test system is based on the concentration dependent inhibitory effect of histamine H_3 agonists on the electrically evoked contractile response of isolated guinea pig jejunum segments as presented in chapter 3 together with an overview of other possible H_3 *in vitro* assays.
From chapter 2 it appears that an imidazole ring, substituted by an alkyl group on the 4(5)-position, is essential for both agonistic and antagonistic activity on the H_3 receptor. Therefore a suitable route was developed for the rapid synthesis of various 4(5)-substituted imidazole derivatives, on a preparative scale. An overview of some potential synthesis routes to these compounds is given in chapter 4.
A new route for the synthesis of 4(5)-(ω-aminoalkyl)-1*H*-imidazoles through C5-lithiation of a suitable 1,2-diprotected imidazole, is described in chapter 5. When the resulting 1,2-diprotected 5-lithio-imidazole is treated with a 1-chloro-ω-iodoalkane, selective substitution of the iodo group takes place and the chloro group of the resultant product was converted into an amino group. These derivatives can be regarded as homologues of histamine and can be used as precursors for a large range of other histaminergic compounds.
In chapter 6, the synthesis and identification of the new, potent and selective histamine H_3 agonist immepip, with a pD_2 value of 8.0 (guinea pig jejunum), is described. Key step in the synthesis of this compound was the addition of 4-pyridinecarboxaldehyde to a suitable 1,2-diprotected 5-lithioimidazole. Immepip can be useful as a pharmacological tool and perhaps as a therapeutical agent, but also, because of its distinctive structure, for SAR- and Molecular Modelling studies.
The influence of alkyl chain length variation on the histamine H_3 receptor activity of a series of histamine homologues was investigated in chapter 7, with an alkyl chain length varying from one methylene group to 10 methylene groups. The ethylene chain of histamine was clearly optimal for H_3 agonistic activity with this series of compounds, since the other compounds were all antagonists (no agonistic activity observed on guinea pig intestine). However, the affinity of the compounds for the

H_3 receptor, increased with increasing chain length, with the pentylene derivative (impentamine) as the most potent and selective H_3 antagonist, with a pA_2 value of 8.4 (guinea pig jejunum). Apparently there is a specific antagonistic binding site for this compound.

The synthesis and activities of a large series of analogues of burimamide are discussed in chapter 8. Within this series the chain length of the alkyl spacer and the *N*-substituents were varied. All analogues were competitive H_3 antagonists. Decrease of the chain length of the alkyl spacer of burimamide led to a decrease in activity, but elongation of the alkyl spacer resulted in more potent and selective H_3 antagonists. The pentylene and hexylene homologues of burimamide are about ten times more potent than burimamide itself. Replacement of the methyl group of burimamide with a variety of substituents, did not have much influence on the affinity for the H_3 receptor. The results indicate a binding behaviour of these derivatives in a non-lipophilic environment, different from other H_3 antagonists, like thioperamide and clobenpropit.

Some rigid H_3 ligands, which can be regarded as analogues of immepip and thioperamide are presented in chapter 9. The analogues of the H_3 antagonist thioperamide, in which the piperidine ring was replaced by the more rigid 1,2,3,6-tetrahydropyridine ring led to a drastic decrease of H_3 antagonistic activity. The replacement of the 4-piperidine ring of the potent H_3 agonist immepip by a 3-piperidine ring results in a weak H_3 antagonist. This information can be very useful for further SAR and molecular modelling studies, since certain conformations of several known potent H_3 agonists (e.g. histamine derivatives and immepip) may be excluded. The 2-piperidine derivative of immepip, which can be regarded as a rigid analogue of histamine, has a negligible affinity for the H_3 receptor. The replacement of the 4-piperidine ring of immepip (**3a**) by a 3-pyrrolidine ring however is allowed and VUF 4864 proved to be a rather good H_3 agonist with a pD_2 value of 7.3, although an intrinsic activity of about 0.8 was observed. Consequently, VUF 4864 (**23**) can also be very useful as a tool for SAR and molecular modelling studies.

For the development of an activation model of the histamine H_3 receptor, cyclopropylhistamine (4(5)-(2-aminocyclopropyl)-1*H*-imidazole) can become a valuable tool, because of its small and rigid structure. It has been reported as a potent H_3 agonist in a patent application. The possible use of cyclopropylhistamine as a template for an H_3 receptor activation model is discussed in chapter 10, together with the synthesis of *trans*-cyclopropylhistamine and the attempts of the preparation of its *cis*-isomer. *trans*-Cyclopropylhistamine proved to be only very weakly active on the histamine H_3 receptor.

Samenvatting

Doordat veel mensen met overgevoeligheidsreacties (zoals huiduitslag, hooikoorts en astma), antihistaminica slikken, wordt histamine vaak in verband gebracht met allergieën. Histamine wordt door het lichaam zelf aangemaakt via decarboxylatie van het aminozuur L-histidine met behulp van het enzym L-histidine decarboxylase. In het lichaam blijken diverse soorten eiwitten (receptoren) aanwezig te zijn, waar histamine een specifieke interactie mee aan kan gaan. Binding van histamine aan deze receptoren heeft een activerende werking en een cascade van specifieke reacties is het gevolg. Tot nu toe zijn er drie typen histamine receptoren geïdentificeerd, nl.:

- de histamine H_1 receptor, die een rol speelt in allergische reacties en waarvan de werking geblokkeerd kan worden door de bovengenoemde antihistaminica (H_1 antagonisten);
- de histamine H_2 receptor die een rol speelt bij de maagzuursecretie en waarvoor diverse maagzuurremmende medicijnen zijn ontwikkeld (H_2 antagonisten) en
- recentelijk is daar de histamine H_3 receptor bijgekomen.

De rol van de histamine H_3 receptor in het lichaam wordt op het ogenblik nog onderzocht. Duidelijk is al wel dat deze receptor een regulerende (remmende) rol speelt in de aanmaak en afgifte van histamine, voorkomend als neurotransmitter in zenuwuiteinden van histaminerge neuronen. De histamine H_3 receptoren zijn voornamelijk gelokaliseerd in de hersenen en blijken tevens een regulerende functie te hebben in de afgifte van andere neurotransmitters. Daardoor lijkt een therapeutische toepassing voor verbindingen die selectief op de H_3 receptor binden en al dan niet de receptor activeren, mogelijk. Op het ogenblik word hierbij voornamelijk gedacht aan medicijnen tegen epilepsie, slaapstoornissen en de ziekte van Alzheimer. Dit moet echter nog grondig onderzocht worden. Hiervoor is het nodig om potente en selectieve verbindingen te ontwikkelen die de histamine H_3 receptor activeren (H_3 agonisten) dan wel blokkeren (H_3 antagonisten).

Het in dit proefschrift beschreven onderzoek heeft zich voornamelijk gericht op het synthetiseren van nieuwe selectieve histamine H_3 actieve verbindingen (H_3 liganden), door middel van structuurmodificaties van bekende H_3 liganden. Omdat alle potente H_3 liganden die tot nu toe beschreven zijn in de literatuur een imidazool ring bevatten met op de 4(5)-positie een alkyl zijketen, hebben we een nieuwe syntheseroute ontwikkeld om verschillende van deze imidazoolderivaten op een snelle manier in handen te krijgen.

Met behulp van deze nieuwe route was het mogelijk om diverse histamine H_3 liganden te ontwikkelen. De H_3 activiteit van de nieuwe verbindingen werd bepaald met behulp van een speciaal voor dit doel ontwikkeld biologisch testsysteem (*in vitro*). Met behulp van de verkregen informatie was het mogelijk om enige relaties op te stellen tussen de structuur van de verbindingen en de H_3 activiteit. Enkele van de beschreven nieuwe potente en selectieve H_3 liganden kunnen mogelijk een belangrijke rol gaan spelen in de farmacologie en eventuele therapeutische toepassingen zijn denkbaar.

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Roel

List of Publications

Publications related to this thesis

- R. C. Vollinga, O. P. Zuiderveld, H. Scheerens, A. Bast and H. Timmerman. A Simple and Rapid *In Vitro* Test System for the Screening of Histamine H_3 Ligands. *Meth. Find. Exp. Clin. Pharmacol.* **14**, 747-751 (1992).
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